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THE PULSE FLOW IN THE BRACHIAL ARTERY*

I. TECHNIC AND GENERAL CONSIDERATIONS

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DEFINITION OF THE PULSE FLOW

In determining the blood-flow to the arm by a method which we have described,[1] the arm is enclosed in a plethysmograph and the venous outflow is obstructed by suddenly raising the pressure in a cuff placed just outside of the instrument. The pressure applied is not sufficiently high to interfere with the free entrance of arterial blood. As a result of this obstruction, the arm begins to swell. The rate of swelling is at first fairly uniform; but later, as the pressure in the blood-vessels rises, there is a falling off in the rate of swelling due, on the one hand, to an escape of venous blood beneath the pressure cuff, and on the other to the force opposed to the entering arterial stream by the rising pressure in the capillaries and arterioles. For a brief period, however, the inflow corresponds to the normal rate of blood-flow to the part.

Tracings of the arm volume obtained by this method always show a series of pulse waves due to the intermittent entrance of blood through the arteries; and just as the average rate of arm swelling indicates the average rate of arterial inflow, so the variations on the tracings occasioned by the arterial pulse indicate the variations of inflow due to each portion of the individual pulse waves. Such tracings may therefore be used to determine how much blood is entering the plethysmograph during each portion of the pulse cycle. Since the point at which the blood enters is at the upper end of the plethysmograph, just above the elbow, and since the main path of arterial inflow at this point is through the brachial artery, the records indicate chiefly the rate of blood-flow at this level of the brachial artery during each portion of the pulse cycle. This may be designated the pulse flow in the brachial artery.

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*From the Department of Internal Medicine, University of Michigan.

*Submitted for publication, March 20, 1913.

1. Hewlett, A. W., and Van Zwaluwenburg, J. G.: The rate of blood flow in the arm. Heart, 1909, i, 87.

TECHNIC

As in our method for determining the blood-flow to the arm, the latter was enclosed in an air plethysmograph which was connected by air transmission with an apparatus which recorded the changes in arm volume. In order to obtain records of the average blood-flow to the arm, any form of volume recorder may be used. We early used the bellows volume recorder of Brodie for this purpose, but more recently we have used a bell recorder similar to that employed for recording respiratory movements. To obtain accurate records of the flow during each portion of the pulse cycle, however, a more sensitive recorder becomes necessary. In a series of exhaustive researches, O. Frank[2] has discussed the necessary requisites of a recorder which shall follow sudden changes in movement with accuracy. The most important of these requisites is that the apparatus itself should possess a rapid rate of inherent vibration. A slow rate of inherent vibration indicates that the apparatus will not follow sudden changes in movement accurately and that the vibrations of the apparatus markedly deform the tracings. With a rate of inherent vibration which is very rapid relative to the changes in movement to be recorded, the latter are recorded much more accurately, and the vibrations of the instrument can be easily recognized should they appear on the tracings.

We have, therefore, endeavored to employ an apparatus which should have as rapid an oscillation time as possible. The following formula furnishes an approximate idea of the factors affecting the time of a single oscillation, T, in a system similar to that which we have employed.

$$T = 2\pi\sqrt{\frac{\frac{L}{A}\underline{d} + M}{\left(\frac{1}{V} + E\right)C}}$$

In this formula, A and L represent, respectively, the cross section and the length of the tube connecting plethysmograph and recorder, and *d* is the specific gravity of the enclosed air. M, which represents the moment of inertia of the recorder, depends on its weight, and the square of the distance through which it is moved. V is the volume of air in the plethysmograph, and E is the rise of pressure produced when one unit of air enters the recording device. The factor C is a constant depending on the atmospheric pressure and the adiabatic correction for air. The above formula is constructed on the assumption that com-

2. Frank, O.: Kritik der elastischen Manometer. Ztschr. f. Biol., 1903, xliv, 445, and a series of articles in the same journal.

pression of air in the transmission tube may be disregarded and that no damping factor is present.[3]

Inspection of the above formula shows that in order to lessen the time of a single vibration in the apparatus the length of the connecting tube, the weight and movement of the recording device and the volume of air in the plethysmograph should be as small as possible; while the cross section of the tube should be as large as possible. In shortening the tube we have endeavored to leave a length which will be sufficient to allow manipulations about the plethysmograph and to allow the individual to move his arm in the suspended plethysmograph without putting traction on the rigidly fixed recorder. For this purpose a length of about 60 cm. is necessary. The connecting tube used had a diameter of 1.2 cm. Between 2,000 and 2,700 c.c. of air usually occupied the portion of plethysmograph about the arm. By far the most important part of the apparatus, however, is the recording device. Two types of sensitive recorders were experimented with. In one of these a soap bubble was placed over the end of the tube leading from the plethysmograph, and the oscillations of its summit occasioned by the volume changes in the arm were photographed on a moving sensitized surface. Garten[4] has published plethysmograph tracings taken by this method and we have attempted to use it for recording the pulse flow in the brachial. After a rather extended trial of the method, however, we became convinced that the oscillation time of the apparatus was inadequate. When connected with a tube sufficiently long to permit fixation of the carrier of the soap bubble the oscillation time was usually between 9 and 13 per second, which was slow for our purposes. Garten's tracings also suggest that the oscillation time in his apparatus was slow, as has been pointed out by O. Frank. The reason for this slow time even when the recorder itself is of neglible weight, becomes evident on inspection of the above formula. In the soap bubble which is essentially a volume recorder the rise of pressure when one unit of air is added to the recorder is practically nil. E, therefore, approaches zero, and since the fraction 1/V is also very small, the period of oscillation is relatively long. We were able to get very satisfactory plethysmograph tracings of the finger by this method, for in this case the volume of air surrounding the finger is small and consequently the fraction 1/V is relatively large. A trial soon convinced us, however, that our method for determining the blood-flow could not be applied satisfactorily to the finger.

3. In the system finally adopted the tube was relatively large and only a very small amount of air entered the recorder. Under such circumstances the compression of air in the tube becomes an important factor which cannot be disregarded. The more complex formula for such a system need not be discussed here.

4. Garten, S.: Ueber ein neues Verfahren zur Verzeichnung von Bewegungsvorgängen und seine Anwendung auf den Volumenpuls. Arch. f. Physiol., 1904, civ, 351.

Since a true volume recorder, even though of negligible weight, necessarily gives slow oscillations when connected with an arm plethysmograph, it became necessary to adopt some other principle for recording the volume changes in the arm. The principle which we adopted was to close the end of the plethysmograph about the arm with unyielding material and to use a Frank capsule as a recorder. The latter is essentially a small Marey tambour in which the recording lever has been replaced by a small mirror which directs an imponderable beam of light on a moving sensitized surface. A description of the apparatus used has been published elsewhere.[5] Since but a very small amount of movement occurs in the Frank capsule during changes in arm volume, the system becomes a pressure system rather than a volume system, and the changes in pressure are recorded by the capsule. By calculating the air in the plethysmograph after the arm is in place, it is possible, however, to graduate the capsule against an equal volume of air in a bottle by allowing measured amounts of water to enter the bottle. The records can thus be read as volume records. With this apparatus high rates of vibration may be obtained. In the tracing shown in Figure 1, for example, the oscillatory frequency of the apparatus was over 100 per second. Some of the tracings in the following articles were taken with an apparatus of much slower vibration time, 15 to 20 per second, but our main conclusions as to the features of the pulse form have been checked by tracings taken with the more sensitive apparatus.

The steps followed in making a series of records were as follows: The volume of arm to be enclosed in the plethysmograph was determined by thrusting it into a large cylinder full of water and measuring the amount of water displaced. The arm was then placed in the plethysmograph, and the primary closure of the end was made by rubber dam. In order to make this closure a rigid one the rubber dam was reinforced by a layer of dentist composition which had been previously softened in hot water. This was applied closely about the arm and over the end of the plethysmograph. While the composition was hardening a large bottle was filled with water until the contained volume of air was equal to the air remaining in the plethysmograph after insertion of the arm. The bottle was then connected with the Frank capsule and the latter was graduated by allowing successive amounts of water, 2 c.c. each, to enter the bottle. The vibration time of the apparatus may then be tested by suddenly adding a small amount of air to the bottle and photographing the after-oscillations of the tambour. The Frank capsule was then connected with the plethysmograph and the apparatus was ready for making records. These latter were made in the same manner as our

5. Van Zwaluwenburg, J. G., and Agnew, J. H.: Some Details of the Auricular Pressure Curves of the Dog. Heart, 1912, iii, 343.

usual records of the blood-flow, except for the form of recorder used. It was found convenient to have an additional tube leading from the plethysmograph in order to regulate the position of the light. This additional tube was left open at the moment that the pressure was applied to the arm, but was closed immediately after. In this way the disturbance that is frequently produced at the moment of applying the pressure was not recorded. Each record taken by the Frank capsule was furthermore checked as to the average blood-flow by a record taken by the usual volume recorder on a moving drum covered by smoked paper.

FACTORS INFLUENCING THE PULSE FLOW

Following Chauveau and Marey, French physiologists usually resolve the blood-flow in an artery into two chief components, the constant and the intermittent flows. Somewhat similarly we have also found it convenient to analyze the pulse flow into two components. The first of these is the average rate of blood-flow in the arm as determined by our ordinary method. The second is the intermittent acceleration or retardation of this flow occasioned by the progress of fluid waves in the artery. These latter are recorded in good plethysmograph tracings if one assumes that the venous outflow does not change materially with each portion of the arterial pulse. Knowing the average rate of blood-flow to an organ it is possible to construct the pulse flow from a good quantitative plethysmographic tracing by erecting on the slant which represents the average flow, verticals for each portion of the plethysmograph curve and connecting their summits. A construction of this type is shown in Figure 1, C. Its identity with the curve obtained directly (Fig. 1, A) is at once apparent.

The velocity in cubic centimeters per unit of time for each portion of the pulse cycle can also be constructed from our pulse flow curves; for the velocity at any moment is the amount of inflow divided by the time. This is represented on each portion of the pulse cycle by the trigonometric tangent to the curve at this point. Constructions of this sort by the method described by Fick[6] are shown in Figure 1, D, and Figure 2, B. These are absolute velocity curves or absolute tachograms in terms of volume flow. They differ from the velocity curves constructed by Fick from plethysmograph tracings and from the tachograms obtained directly by V. Kries,[7] in that they represent absolute volume velocities in the brachial artery and not simply changes in velocity which are superimposed on an unknown constant. If one knew the exact diameter of the brachial artery these volume velocities might be converted into linear velocities. The close resemblance of our volume velocity constructions

6. Fick: Gesammelte Schriften, Würzburg, 1904, iii, 550.
7. Kries, V.: Studien zur Pulslehre. Freiburg, 1892.

to the variations in linear velocity determined directly on animals may be seen by comparing our construction with the velocity curves frequently published in standard works in physiology.[8] We see no advantage, however, in converting our pulse flows into pulse velocities.

Not only may the pulse flow be resolved into the two components, average flow and wave motion, but the two appear to us to depend on factors which are more or less independent of one another. The average rate of blood-flow in an artery depends in the main on the average arterial pressure and the amount of resistance offered to the flow of blood through the arterioles and capillaries supplied by the artery under consideration. A limited area of vasodilation will greatly accelerate the local blood-flow although it may have but little effect on the general level of arterial pressure. The size and form of the pulse waves as distinguished from the constant flow may, theoretically at least, vary widely even though the flow remains constant. The pulse waves depend primarily on the systolic output from the heart and it is to be expected that they will vary with variations in the amount of blood expelled from the heart and with the character of the expulsion, rapid, slow, etc. But the volume of the pulse entering any particular arterial trunk is also dependent on the relative size of this trunk. If for example the main artery leading to a part widens and the conditions throughout other parts of the circulatory apparatus remain unchanged, a more voluminous wave will enter this artery even though the size of the arterioles and capillaries which drain it remain unchanged. The pulse waves are also greatly modified by their passage through the arterial tree. Changes in the elasticity of the arterial walls, the tendency of the arteries to impart their own oscillation times to waves coming to them, the reflection of waves at the periphery, all these render the finer analysis of the peripheral pulse a matter of great complexity.

Thus far we have not used the above method on animals. Nor can it be confidently predicted to what degree our results on the arm of man can be transferred to the pulse flows in other organs or to the flow in the extremities of other animals. It must be remembered that the pulse flow in each organ is dependent more or less on local vascular conditions. Variations in the size of the main artery leading to the part, in the elasticity of this artery, in its length and distance from the heart, and in the condition of the finer arterioles and capillaries will necessarily modify the picture of the pulse flow. We know that in the arm of man the blood-flow usually maintained is far short of that which is possible under special conditions, such as local muscular exercise or the local or general application of heat. So far as we know, no studies of the absolute pulse flow in an artery as distinguished from the linear velocity had appeared

8. Marey, E. J.: La circulation du sang, Paris, 1881, Fig. 188.

previous to our preliminary report.[9] Since that time, Hürthle[10] has recorded the pulse flows in the arteries of the dog by means of a specially constructed *Stromuhr*. His tracings obtained from the carotid and the femoral arteries differ considerably from those which we have obtained from the arm of man, especially in regard to the finer waves present. The differences between our tracings and those of Hürthle are doubtlessly due in part to the difference in the arteries selected for study. Aside from this difference in the material used, however, it is evident from Hürthle's statements that his instrument was far less sensitive than was ours to rapid variations in the blood-flow, and in particular he states that on account of its lack of sensitiveness reflected waves may escape record by the *Stromuhr* which he used. From our studies we believe that reflected pulse waves are of great importance in certain types of the brachial pulse flow in man and that any method which fails to record these accurately misses one of the most interesting and important features of the pulse flow.

In the following articles certain types of marked modification of the pulse flow in the brachial artery will be presented, and we shall attempt to explain the causes of these modifications in accordance with the above considerations, so far as this seems possible. It is not to be expected that our explanations will be free of error, but we hope, at least, that our objective records will throw light on some of the complex problems of the peripheral arterial pulse of man.

II. RELATION TO THE AVERAGE BLOOD-FLOW. EFFECT OF NITROGLYCERIN

A. W. HEWLETT, J. G. VAN ZWALUWENBURG AND J. H. AGNEW
ANN ARBOR

GENERAL FORM OF THE PULSE FLOW

As a rule the pulse flow in the brachial artery follows a fairly uniform course. There is a sudden and marked increase in the flow when the main pulse wave arrives from the heart (Figs. 1 and 3). Immediately following this primary wave there is a momentary slowing in the flow, which sometimes becomes a momentary backward movement of the blood in the artery. This is interrupted by the positive dicrotic wave, after which there is, as a rule, comparatively little movement in the column of blood in the artery up to the entrance of the next primary wave from the heart. What little movement takes place during diastole is usually in

9. Hewlett, A. W.: Van Zwaluwenburg, J. G., and Agnew, J. H.: A New Method for Studying the Brachial Pulse of Man. Tr. Assn. Am. Phys., 1912, xxvii, 188. Read May 14, 1912.

10. Hürthle, K.: Ueber die Beziehung zwischen Druck und Geschwindigkeit des Blutes in den Arterien. Arch. f. Physiol., 1912, cxlvii, 525.

the positive direction, although it seems possible to have a stationary column of blood or even a slight continuous backward movement toward the heart during diastole without pathological significance. In addition to the main waves described above, there are frequently finer oscillations on the tracings other than those occasioned by movements on the part of the patient.

RELATION BETWEEN THE PRIMARY PULSE WAVE AND THE BLOOD-FLOW

It has been pointed out in the preceding article that the pulse flow may be resolved into two components, the average blood-flow to the part and the variation in this flow produced by the arterial pulse waves. Of these waves the primary pulse wave is the largest and most important, and one of the first questions that arises is whether any relation exists between the size of the primary wave and the average rate of blood-flow to the arm. May one, in other words, draw any conclusion as to the rate of blood-flow in the arm from the size of the volume pulse? This question has been tested in two ways: first, by comparing the blood-flows and the volume pulses of different individuals under fairly uniform conditions, and second, by comparing them in a single individual under varying conditions. In the following table we have recorded the average rates of blood-flow in a number of individuals together with the heights of the primary pulse waves, as measured above the average blood-flow.

TABLE 1.—RELATION BETWEEN HEIGHT OF PRIMARY WAVE AND RATE OF BLOOD-FLOW IN DIFFERENT INDIVIDUALS

Name	Diagnosis	Height of primary waves in c.c.	Total amount of blood entering the arm in a complete pulse cycle in c.c.	Average blood-flow in c.c. per 100 c.c. of arm substance per minute
Bis	Patent ductus arteriosus	0.66	0.4	1.7
Cr	Neurasthenia	0.6	0.5	1.8
Fli	Rheumatism	0.4	0.4	2.3
Eas	Arthritis	0.9	0.7	2.6
McQu	Aneurism	0.5	0.5	3.2
Smi	Pleurisy	0.5	0.7	3.6
Gui	Diabetes and arteriosclerosis	0.95	0.7	3.8
Hic	Hypertension	0.6	0.8	4.5
Mus	Hypertension	0.6	1.2	4.6
Haz	Hypertension	0.6	0.9	5.4
Cha	Pernicious anemia	0.45	0.8	5.7
Mor	Pernicious anemia	0.6	1.5	6.0
Fly	Hypertension	0.9	1.1	6.1
Stem	Hypertension	0.7	1.0	6.2

It is evident from this table that in different individuals the size of the volume pulse gives no indication of the rate of blood-flow to the arm.

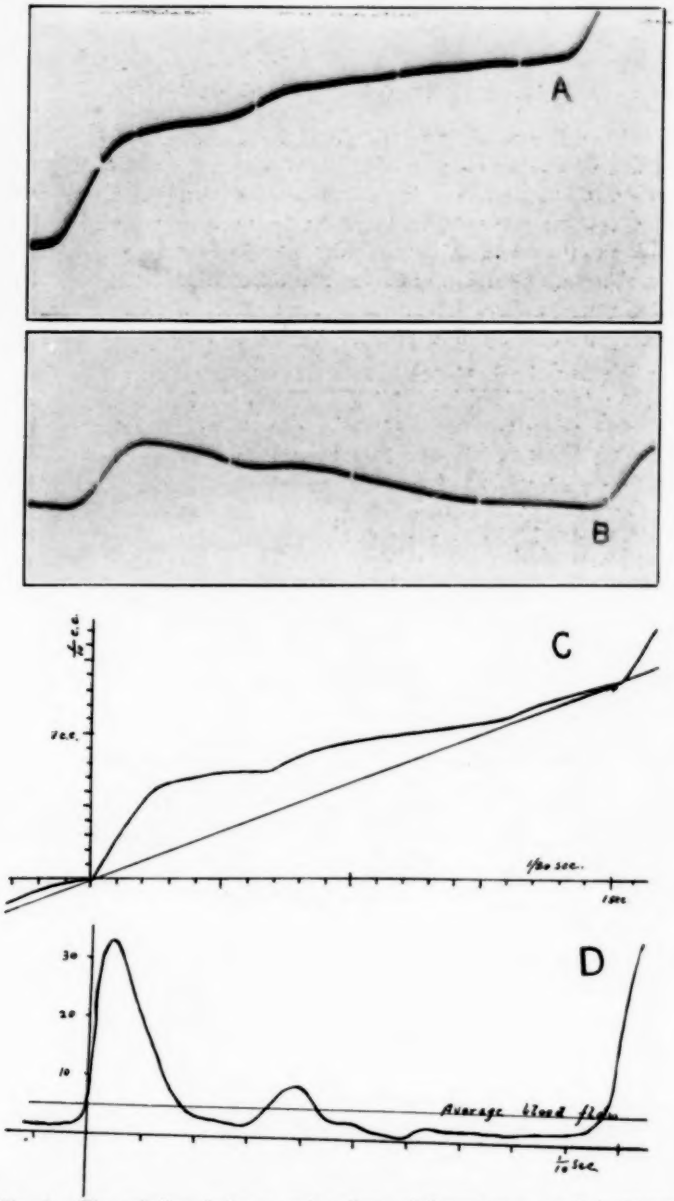

Fig. 1.—The relation between pulse flow, plethysmogram, and tachogram. Instrumental oscillations 106 per second. A is the pulse flow tracing. B is an ordinary plethysmogram taken from the same individual when the venous outflow from the arm was not obstructed. C is a construction of the pulse flow from the plethysmogram and from the average flow of blood as determined by our usual method. D is a velocity construction or tachogram. This was constructed from A by erecting trigonometric tangents of the curve on an abscissa representing the time. In all of our figures the horizontal interruptions of the tracings represent differences of volume of 2 c.c. and the vertical interruptions are produced by a time marker. Two vertical interruptions represent one-half second. The average rate of blood-flow was 5 c.c. per 100 c.c. of arm substance per minute. The primary wave put in 0.46 c.c. above the average rate of flow. The total amount of blood entering the arm with one complete pulse cycle was 1.35 c.c.

The blood flow to the arm may be markedly influenced by the room temperature[11] and by exercise of the arm within the plethysmograph.[12] In one set of experiments a single individual was subjected to a variety of room temperatures. When the room temperature was agreeable the blood-flow to the arm usually ranged between 3 c.c. and 6 c.c. per 100 c.c. of arm substance per minute; during marked chilliness it fell to 1.5 c.c., and during uncomfortable warmth it rose to 13.7 c.c. During agreeable room temperatures the flow pursued the course already outlined. The

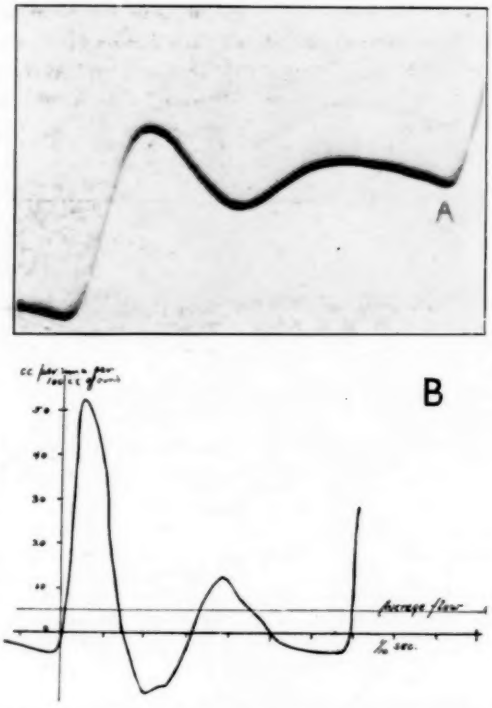

Fig. 2.—The pulse flow and the constructed tachogram in a typical nitroglycerin pulse. From the same individual as Figure 1. Instrumental oscillations 106 per minute. Note the increase in the size of the primary wave, the marked negative wave immediately following, and the elimination of smaller secondary waves. Average rate of blood-flow 5 c.c. per 100 c.c. of arm substance per minute. The primary wave put in 1.3 c.c. above the average rate of flow. The total amount of blood entering the arm in a complete pulse cycle was 1.1 c.c.

11. Hewlett, A. W.: The Effect of Room Temperature upon the Blood Flow in the Arm, with a few Observations on the Effect of Fever. Heart, 1911, ii, 230.

12. Hewlett, A. W., and Van Zwaluwenburg, J. G.: The Rate of Blood Flow in the Arm. Heart, 1909, i, 87.

primary wave delivered from 0.45 to 0.9 c.c. of blood measured above the average rate of inflow, and there was comparatively little movement in the column of blood from the end of one primary wave to the beginning of the next. When the individual was chilled and the blood-flow to the arm was reduced to a minimum, there was usually a corresponding reduction in the size of the primary pulse wave so that the main inflow to the arm still occurred during the early part of systole, and from then on the flow practically ceased. There was, however, a tendency for the reduction in flow to be somewhat greater than the reduction in the primary wave, which resulted, in some tracings, in a tendency to a slight back-flow of blood between the primary pulse waves.

When the room was heated and the flow of blood was markedly increased, there was but a moderate increase in the height of the primary pulse wave as measured above the average rate of blood-flow. The flow

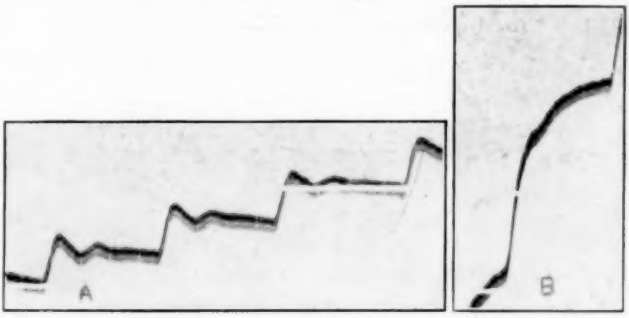

Fig. 3.—The effect of a marked change in the blood-flow on the pulse flow. Instrumental oscillations 46 per second. A. The ordinary flow was 2.4 c.c. per 100 c.c. of arm substance per minute. The primary wave was large relative to this flow. A part of the primary wave was immediately reflected during the systolic portion of the pulse and the tracing shows a slow backward flow during diastole. B. Following exercise of the arm within the plethysmograph the flow was increased to about 20 c.c. per 100 c.c. of arm substance per minute. The flow in the brachial artery more nearly approaches a straight line.

between primary waves was, however, markedly increased. Instead of the step-like entrance of blood into the arm which characterizes the usual flow into the brachial artery, there was now more of a continuous flow throughout all parts of the pulse cycle. This same type of tracing was also obtained in the rapid flow following exercise of the muscles within the plethysmograph (Fig. 3), and it was occasionally encountered in patients with unusually rapid rates of flow. The explanation of this change in the form of the pulse flow appears obvious. Ordinarily the amount of blood delivered to the arm by the primary pulse wave is about equal to that which is allowed to escape through the finer arterioles

during the entire pulse cycle. When, however, a very rapid flow of blood through the part is demanded, the arterioles relax and the large amount of blood passing through them can no longer be supplied by the primary pulse waves. Consequently the flow in the brachial artery becomes more or less continuous.

It is apparent, therefore, that even in a single individual the size of the volume pulse is no accurate guide to the rate of blood-flow through the arm. This is especially true of very rapid flows, for in these the rate of flow is always increased to a far greater extent than is the size of the primary waves. We shall return to this lack of parallels between the blood-flow and the size of the primary waves in the discussion of the nitroglycerin pulse, where very large primary waves are associated with relatively slow flows (Table 2).

TABLE 2.—Relation Between Height of Primary Wave and Rate of Blood-Flow in a Single Individual With and Without Nitroglycerin

Without Nitroglycerin			With Nitroglycerin		
Height of Primary Wave in c.c	Total Blood Entering in One Pulse Cycle in c.c.	Average Flow in c.c Per 100 c.c. of Arm Per Minute	Height of Primary Wave in c.c	Total Blood Entering in One Pulse Cycle in c.c.	Average Flow in c.c. Per 100 c.c. of Arm Per Minute
0.36	0.41	1.5	0.86	0.33	1.5
0.26	0.50	2.0	1.09	0.35	1.7
0.77	0.72	3.1	1.10	0.52	2.1
0.96	1.47	6.3	1.06	0.99	4.6
0.95	1.48	6.9	0.95	1.14	5.4
0.90	2.5	13.7	1.20	1.34	6.0
			1.12	1.53	7.1

NEGATIVE OR BACK-FLOWS IN THE BRACHIAL ARTERY

Indications of backward movement of the blood-column in the brachial artery are frequently encountered in our tracings. These backward movements appear to be of two varieties. In the first the flow is of short duration and of wave-like character. This type of negative flow is most apt to occur during the late systolic period of the pulse, immediately after the primary wave. As it is particularly well marked in the nitroglycerin pulse its discussion will be deferred until that pulse is considered.[13]

The other type of backward movement is of a more continuous character and extends from the dicrotic wave to the beginning of the next primary wave. This continuous, diastolic backward flow is particularly

13. For convenience we shall speak of the systolic period of the pulse as the portion preceding the beginning of the dicrotic wave, although we are aware that this may not exactly represent the period of ventricular systole.

marked in many tracings from aortic insufficiency, but it is occasionally indicated even in normal individuals (Fig. 3). Although tracings from normal individuals occasionally show this continuous diastolic back-flow, the accuracy of these tracings might be questioned. The retrograde movement on the tracings is never very marked and should our records underestimate the true blood-flow by 10 or 20 per cent., then the supposed negative flow would usually disappear. We are inclined to believe, however, that a slight continuous diastolic back-flow may occasionally occur in the brachial artery, even in normal individuals. Theoretically, at least, this seems possible. The flow between the primary pulse waves depends on the relation which exists between the amount of blood put into the brachial artery by the primary wave and the amount escaping through the arterioles during the entire pulse cycle. The latter is proportional to the average blood-flow. If the average flow is fast relative to the size of the primary waves then the flow between beats will be positive and rapid, as is seen after local exercise (Fig. 3). If, on the other hand, the average flow is relatively slow, then the flow between beats may be reduced to zero or even to a negative quantity (Fig. 3). These two factors, average flow and size of primary waves, are certainly more or less independent of each another. The former depends in the main on the blood-pressure and the resistance encountered in the smaller arterioles and capillaries. The latter depends on various factors, especially the amount of ventricular systole and the size of the local arteries. If, for example, the finer arterioles become constricted so as to cut down the average blood-flow and at the same time the systolic output from the heart and the size of the larger arteries leading to and in the arm are not affected, then a large pulse wave will enter the brachial artery. Not all of this blood can escape into the capillaries and veins during the pulse period, and some will, therefore, be returned toward the aorta between pulse beats. Apparently this return may be in the form of a sudden wave late in systole or as a more continuous flow during diastole or as a combination of the two. Such a return of blood from the brachial artery need cause no rise in the general arterial pressure, for the flow through other organs may be so free as to counterbalance the local resistance encountered in the arm.

THE NITROGLYCERIN PULSE

Nitroglycerin, even in such small doses as one drop of the 1 per cent. alcoholic solution placed on the tongue, usually produced marked changes in the form of the peripheral pulse. These changes begin in two or three minutes, reach their maximum in five or six minutes, and pass off in fifteen minutes or more. The radial pulse becomes larger and fuller, and the pulsations recorded by the Erlanger sphygmomanometer become

larger.[14] Plethysmograph records of the arm usually show arm swellings of 15 c.c. or more, amounts which are of the same order of magnitude as those produced by the brief applications of heat or cold to distant parts of the skin.[15] The plethysmograph pulse becomes distinctly larger. Changes in the blood-flow to the arm also occur, but we have found the flows to be sometimes faster and sometimes slower, and on the whole, they are rather difficult to follow. The marked increase in blood-flow which might be anticipated from the arm swelling and from the increase in the size of the volume pulse is not realized.

This lack of parallelism between the arm swelling and the local blood-flow is noteworthy, because it is often assumed that a rapid increase in the size of an organ is trustworthy evidence of an increase in the local blood-flow. Such a parallelism seems to exist when the arm is subjected to thermic influences,[16] but it is certainly absent in nitroglycerin action. This disproportion between arm swelling and blood-flow may occur even when the systolic blood-pressure is but slightly reduced, and we have attributed it to a dilatation of the arterial trunks without a corresponding relaxation of the arterioles which are believed to govern the local flow. The somewhat similar observation of Bunch[17] that the submaxillary gland may decrease in size even though the local blood-flow increases, has been held to be due to a reduction in the size of the gland by loss of secretion.

The changes in the brachial pulse flow produced by nitroglycerin are so characteristic, that in going over a set of records, the nitroglycerin tracings can usually be recognized with ease. Their main features, readily seen by comparing Figure 2, A with Figure 1, A, are the large size of the primary wave, the marked negative wave immediately following, the large dicrotic wave, and the elimination of other secondary oscillations. In the individual particularly studied, the primary pulse waves at the height of the drug action were always very large, equalling or exceeding the primary waves which accompanied the most rapid blood-flows caused by a warm room. (See Table 2.) Even when the flow of blood through the arm was greatly slowed by a chilly room, the primary waves of the nitroglycerin pulse remained large.

Two explanations of the large nitroglycerin pulse seem possible. On the one hand it is conceivable that this might depend on the manner in

14. Hewlett, A. W.: The Effect of Amyl Nitrate Inhalations upon the Blood-Pressure in Man. Jour. Med. Research, 1906, xv, 383.

15. Müller, O.: Ueber die Blutverteilung im menschlichen Körper unter dem Einfluss thermischer Reize. Deutsch. Arch. f. klin. Med., 1905, lxxxii, 547.

16. Hewlett, A. W., Van Zwaluwenburg, J. G., and Marshall, Mark: The Effect of Some Hydrotherapeutic Procedures on the Blood-Flow in the Arm. THE ARCHIVES INT. MED., 1911, viii, 591

17. Bunch, I. L.: On the Changes in Volume of the Submaxillary Gland During Activity. Jour. Physiol., 1900, xxvi, 1.

which the blood is expelled from the heart. A large systolic output or a sudden emptying of the ventricle against a low arterial pressure might cause a pulse wave of unusual height, which was propagated as such into the brachial artery. On the other hand, the large pulse wave might be due to a relaxed condition of the larger vessels in the arm. Animal experiments[18] have shown that nitroglycerin increases the systolic output in some dogs, but not in others. The effect on the cardiac output in man is not known, for conclusions drawn from changes in pulse pressure are open to numerous criticisms.[19] On the other hand, there is positive evidence in favor of the view that the nitroglycerin changes in the brachial pulse of man are due to peripheral influences, for we have repeatedly found that carotid tracings taken by tambours do not show these changes even when the brachial tracings do (Fig. 4).

Carotid tracings are fairly representative of the central aortic pulse, and we are, therefore, inclined to attribute the characteristic changes

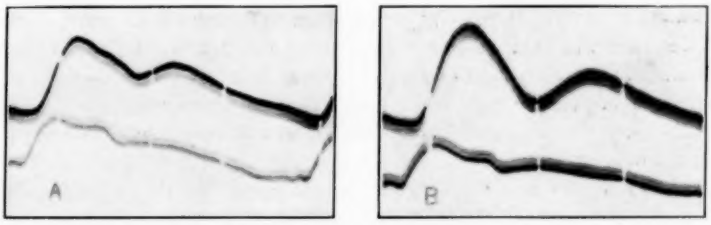

Fig. 4.—Pressure tracings from the carotid artery (above) and from the brachial artery (below), before (A) and after (B) the administration of nitroglycerin. Note that the brachial was affected to a far greater extent than the carotid.

of the nitroglycerin pulse to changes in the peripheral arteries. A relaxed condition of the arterial trunks leading to and distributed in the arm would permit a large undamped wave to enter the brachial artery. Direct evidence in favor of the arterial relaxation is found in the obliterations of the smaller arterial oscillations, for Veiel[20] and Müller and Weiss[21] have shown that these tend to disappear from the pressure pulse of the radial artery when the tonus of the larger arterial trunks is diminished.

18. MacNider, W. de B.: The Action of the Nitrites on the Heart. Am. Jour. Med. Sc., 1908, cxxxv, 99.

19. Müller, O.: Der arterielle Blutdruck und seine Messung beim Menschen. Ergebn. d. inn. Med. u. Kinderh., 1908, ii, 367.

20. Veiel, E.: Ueber die Bedeutung der Pulsform. Deutsch. Arch. f. klin. Med., 1912, cv, 250.

21. Müller, O., and Weiss, E.: Ueber die Topographie, die Entstehung und die Bedeutung des menschlichen Sphygmogrammes. Deutsch. Arch. f. klin. Med., 1912, cv, 320.

PERIPHERAL REFLECTION OF THE PRIMARY PULSE WAVE

The brief negative wave of late systole which sometimes follows the primary wave even on normal tracings is very markedly accentuated in the nitroglycerin pulse. There seems to be no possibility of attributing this negative wave to an experimental error, for it is much too prolonged to be due to a defect in the recording apparatus, and the negative flow is far too rapid to be explained as due to any error in the estimation of the average blood-flow. To our minds no explanation for this negative wave seems possible, other than that it is due to a momentary and marked wave of back-flow in the brachial artery. Although the velocity tracings that have been taken directly from animals are open to the criticism, that the instruments used were not especially adapted for recording sudden changes in movement, nevertheless, it is interesting to note that in some of these[22] a negative velocity has been recorded just following the marked positive velocity produced by the primary pulse wave. Furthermore, in v. Kries' tachograms, as well as in one published by O. Frank,[23] the greatest reduction in velocity occurred just after the main wave, and v. Kries[7] has shown that this reduction of velocity is particularly well marked in certain stages of amyl nitrite action. These records of absolute and of relative pulse velocities, therefore, agree with our tracings of the pulse flow in the brachial artery of man in showing that a negative wave in the artery might be expected just following the main pulse wave.

The question arises as to whether this negative wave in the brachial artery is of central or peripheral origin. If of central origin, its explanation would be that frequently given for the dicrotic notch, viz.: a negative wave originating at the beginning of the aorta and produced by the settling back of the blood-column against the semilunar valves. There can be no doubt that a negative wave of this character does originate at the base of the aorta, but it is too small to explain the marked back-flow which occurs in the brachial arteries during the action of nitroglycerin, for O. Frank[24] has shown that the closure of the valves produces only a brief notch in the aortic pressure tracing. The negative flow wave in the brachial artery, on the other hand, may amount to 0.5 c.c., and to over 50 per cent. of the primary wave after giving nitroglycerin. We have, therefore, concluded that in the brachial artery the negative wave of late systole is due in the main to peripheral influences.

There has been considerable discussion among physiologists as to the degree to which peripheral reflection of pulse waves may affect the form of the pulse. It is well recognized that whenever a liquid wave travels along an elastic tube of great length, it will be in part reflected as a

22. Marey, E. J.: La circulation du sang, Paris, 1881, Fig. 188.

23. Frank, O.: Konstruktion und Theorie eines neuen Tachographen. Ztschr. f. Biol., 1907, l, 303.

24. Frank, O.: Der Puls in den Arterien. Ztschr. f. Biol., 1905, xlvi, 441.

positive wave whenever an obstruction is met, and that it will be in part reflected as a negative wave whenever the tube suddenly widens. The resultant of such a combination of reflected positive and negative waves from numerous points in the arterial tree must be extremely complicated, and attempts to explain variations in the pulse form from this point of view have not led to very satisfactory conclusions. O. Frank[24] has, therefore, proposed that the subject be viewed from a somewhat different point of view. He has shown that in the central aortic pulse there is little evidence of the presence of waves reflected from the periphery. On the other hand, the femoral pulse of the dog differs markedly in type from that of the aorta. There is not simply a smoothing out of the general features of the aortic pulse such as one might expect from the passage of a wave along a long elastic tube, but the dicrotic notch is deepened, the pulse pressure is markedly increased and the primary wave may show a higher systolic pressure than actually exists in the aorta. Dawson[25] has also shown that the maximum pressures may be higher at certain parts of the arterial tree than at the root of the aorta. In order to explain his findings, O. Frank has compared the pulse in the peripheral arteries with the pressure records obtained by a manometer of relatively slow vibration period. Such a manometer will deform the original pulse picture by imparting its own oscillation time to the waves, and it is probable that the elastic arteries will have a similar effect on the pulse which comes to them. According to Frank, the wave motion in the arteries is more readily understood if it be compared with the stationary waves of a relatively short closed elastic system than if it be compared to the waves which travel along elastic tubes of great length.

The hypothesis which we have adopted to explain the marked negative flow toward the end of the systolic period of the nitroglycerin pulse is in accord with Frank's views. This hypothesis is as follows: When nitroglycerin is given a large primary wave enters the relaxed brachial artery and passes down its branches; but since only a small portion of this wave can escape through the unrelaxed arterioles and capillaries, the larger arterial trunks and their branches become distended, and through their elastic recoil the wave is returned toward the heart. According to this hypothesis, the immediate reflection of the primary wave from various points in the arterial tree becomes of less importance than the reflection by the distention and elastic recoil of the arterial walls. To make the conception more clear, one may compare the reflection of pulse waves with the reflection of an air wave, which enters a resonator of the general type of a flask with a relatively narrow neck. When a sudden wave of air is thrown into such a flask it is not immediately reflected as

25. Dawson, P.: The Lateral Blood "Pressures" at Different Points of the Arterial Tree. Am. Jour. Physiol., 1906, xv, 244.

it would be from a plane surface, but it causes a compression of the air within the flask, and this is reflected by the "elastic recoil" of the compressed air. Its period of oscillation is far longer than if the wave had entered a closed pipe which did not open out into a flask. We believe, therefore, that the reflection of the primary wave from the periphery, which is such a prominent feature of the nitroglycerin pulse, is due in the main to an elastic recoil of the larger arteries and their branches.

Even under normal circumstances a negative wave of blood-flow in the brachial artery frequently succeeds the primary pulse wave. We believe that this is to be explained on the same principle that we have used to explain the occurrence in the nitroglycerin pulse; viz., by a reflection of the main pulse wave due to the elastic recoil of the arteries. The peculiar features of the nitroglycerin pulse may all be explained by assuming a reduction in the tonus of the larger arteries and their branches.

CONCLUSIONS

1. An augmentation of the arm volume does not necessarily indicate an increase in the local blood-flow. This is seen in the nitroglycerin pulse.

2. The volume pulse is no reliable index of changes in the blood-flow through the arm. In different pathological conditions the two bear no relation to each other. In a single individual the volume pulse is relatively small when very fast flows are produced by heat and by exercise, and it is relatively large after nitroglycerin has been given.

3. A slow back-flow toward the heart during the diastolic period of the pulse is indicated in some of our normal tracings of the brachial pulse flow. This is not common, however, and it may be due in some cases at least to technical errors.

4. A peripheral reflection of the primary pulse wave due to the elastic recoil of the peripheral arteries may produce a negative wave of flow in the brachial artery during late systole. This is a very prominent feature of the nitroglycerin pulse.

III. AORTIC INSUFFICIENCY

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In some patients with physical signs of aortic insufficiency the arterial pulse shows but few changes from the normal. In its typical form, however, the arterial pulse of aortic insufficiency is characterized by a large and sudden rise (*pulsus magnus et celer,* water hammer pulse) and by a rapid fall (collapsing pulse). The arteries are often tortuous and of large caliber. The diastolic pressure is low and the difference between sys-

tolic and diastolic pressures is markedly increased; so that these changes in the pressures constitute an important sign of the disease. In typical cases the sphymogram of the radial pulse is unusually large. According to Mackenzie and Broadbent the most characteristic feature of the radial pulse is the rapid fall which takes place before the occurrence of the dicrotic notch. Stewart[26] had pointed out that since the dicrotic notch marks the end of ventricular systole this fall previous to the notch cannot depend directly on a regurgitant wave into the ventricle. Mainly on the basis of animal experiments he has advanced the view that there is a reflex inhibition of the vasomotor center which increases the flow through the capillaries and so lowers the blood-pressure even during the latter part of the cardiac systole. According to Stewart, the amount of blood which regurgitates into the ventricle is inconsiderable and the fall of diastolic pressure is mainly dependent on the increased flow through the capillaries. MacCallum[27] has studied the effect of aortic regurgitation on the pulse form and the pressures when an artificial (rubber) aorta is connected to the living heart of a dog. His measurements indicate that while the amount of blood which regurgitates into the ventricle in artificially produced aortic insufficiency is small when expressed in cubic centimeters it may be large relative to the ventricular output. MacCallum's method of experiment enabled him to measure directly the outflow from his artificial aorta and he was thus able to demonstrate that the pulse form and pressure changes characteristic of aortic insufficiency did not in his experiments depend on an increased outflow. As a result of these experiments, he concluded that in aortic insufficiency the regurgitation of blood into the ventricle produced the low diastolic pressure, that the extensive and violent excursions of the ventricle produced the large pulse pressure, and that the low diastolic tension of the arterial wall allowed a large systolic "fling" which accounted for a large part of the high pulse wave and for the low position of the dicrotic notch. It seems to us that the objections which might be raised to MacCallum's experiments relate mainly to the "fling" which he describes. Did this fling in his tracings originate in his recording apparatus or in the rubber tube used to replace the aorta, and if it originated in the latter, may one assume a similar "fling" in the arterial system of man?

We have approached this subject from the study of the brachial flow pulse as it occurs in the aortic insufficiency of man. It is true as MacCallum has pointed out that in man the conditions may be modified by secondary changes in the arteries themselves, and that the characteristic

26. Stewart, H. A.: Experimental and Clinical Investigation of the Pulse and Blood-Pressure Changes in Aortic Insufficiency. THE ARCHIVES INT. MED., 1908, i, 102. (Literature reviewed.)

27. MacCallum, W. G.: The Changes in the Circulation in Aortic Insufficiency. Bull. Johns Hopkins Hospital, 1911, xxii, 197.

pulse in man may not be due entirely to the valve lesion. However, since the chief interest in the subject centers about the collapsing pulse as observed clinically, it is of prime importance that one should obtain a clear conception of the collapsing pulse encountered in man. The following questions therefore present themselves: Is there an appreciable backflow in the brachial artery during diastole in typical instances of the collapsing pulse? Is the fall of pressure which has been recorded during the latter part of systole due to a rapid escape of blood through the capillaries or is it due to an arterial "fling"?

THE PRIMARY WAVE IN COLLAPSING PULSES

The following study of the brachial pulse flow in aortic insufficiency is based on tracings obtained from seven patients who showed all degrees of the vascular phenomena characteristic of this lesion. This list includes all patients showing marked vascular phenomena who presented themselves at the medical clinic during the past eight months, but omits four or five with diastolic murmurs unaccompanied by definite vascular phenomena, for the reason that little change from the normal was to be expected in such cases. In the following table the cases are arranged according to the size of the primary pulse waves in the brachial artery as measured above the average inflow.

TABLE 3.—PRIMARY WAVE IN COLLAPSING PULSES. CASES ARRANGED ACCORDING TO SIZE OF PRIMARY PULSE WAVES IN THE BRACHIAL ARTERY AS MEASURED ABOVE AVERAGE INFLOW

Name	Collapsing Quality	Capill. Pulse	Duroziez's Sign	Brachial Pressures	Area Heart Shadow	Height Primary Wave	Flow Per Beat	Flow per 100 c.c. Arm Per Minute
Hol. ...	+	++	+	150	...	2.5	2.0	10.0
Nei.	+	++	+	138-25	123	2.1	0.5	2.3
Wid. ...	+	+	+	142-45	134	2.0	0.5	3.3
Wood ..	+	+	+	120-48	118	1.4	0.5	2.3
Br.	+	+	+	132-50	...	1.4	1.1	9.3
Wel. ...	?—0	0	0	.....	121	0.8	1.0	3.1
Cl.	0	0	?	158	116	0.6	0.4	3.8

It will be seen that in five of the seven cases the vascular phenomena were clinically well marked and in two they were absent or slight. In all of the former the primary wave equalled or exceeded 1.4 c.c. of blood. This large primary wave is characteristic of the marked vascular phenomena of aortic insufficiency, for in no other condition have we encountered primary waves which exceeded 1.1 c.c., while the usual size of the primary wave is from 0.4 to 0.9 c.c. The causes of a large primary wave have already been enumerated in the discussion of the large wave of the nitroglycerin pulse. In brief they are (1) a large or sudden systolic output from the heart, and (2) a relaxed condition of the

peripheral arteries which allows a large pulse wave to enter them. Both factors probably play a part in the production of the unusually large primary wave which seems to be characteristic of collapsing pulses.

THE DIASTOLIC BACK-FLOW IN THE BRACHIAL ARTERY

We have already mentioned the fact that a slight continuous back-flow during the diastolic period of the pulse may be indicated by our tracings from normal individuals. This is rather uncommon, however, and it is always a slow movement. In the case of the collapsing pulse, the diastolic back-flow may be very rapid, and in some of our tracings three-fourths of the blood which entered the brachial during the primary wave left again during the remainder of the pulse cycle (Fig. 5). The actual amount of back-flow depends in part on the amount of blood which is allowed to pass through the arterioles and capillaries into the veins.

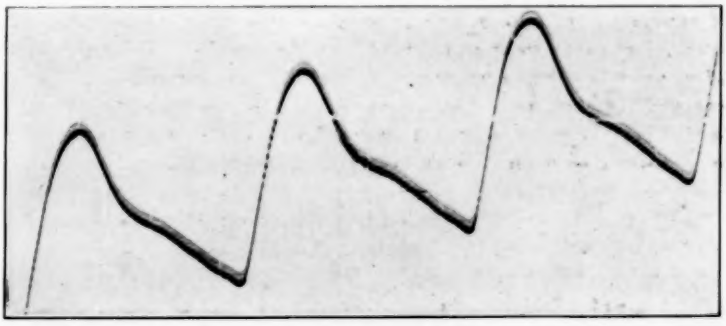

Fig. 5.—The pulse flow in a markedly collapsing pulse of aortic insufficiency. Average blood-flow 3.6 c.c. per minute. The primary wave put in 2 c.c. above the average rate of flow. The total amount of blood entering with one complete pulse cycle was 0.5 c.c.

Should the peripheral resistance in the arm be reduced then a large part of the blood which enters with the primary wave may pass on into the capillaries and veins, leaving but little to be returned toward the heart in diastole. Such a condition is not the rule, however, and it is certain that the collapsing character of the pulse has nothing to do with the amount of resistance offered by the local arterioles and capillaries of the arm. It was present whether the blood-flow in the arm was rapid of slow (Table 3).

It is possible to demonstrate in another way that the diastolic fall of pressure in aortic insufficiency does not depend on a rapid flow through the capillaries of the arm. Owing to the low diastolic pressure in this condition, a relatively low pressure must be used in the cuff that we use to constrict the veins just outside of the plethysmograph. In aortic

insufficiency pressures of 40 to 70 mm. of mercury may usually be used without markedly deforming the flow tracings. When higher pressures (70 to 120 mm.) are used, an interesting deformity appears on the tracings. As will be seen in Figure 6, the result is a reduction in the height of the primary wave and a coincident reduction in the back-flow during the latter part of the pulse tracing. The rate of blood-flow to the arm remains practically unchanged. The higher pressure in the cuff has cut off the arterial inflow and arterial outflow to about the same extent. Had the marked fall in diastolic pressure been due to a rapid flow through the arterioles and capillaries of the arm, one would have anticipated that a rise of pressure in the cuff above the diastolic pressure would have interrupted this onflow and have slowed the rate of flow to the arm.

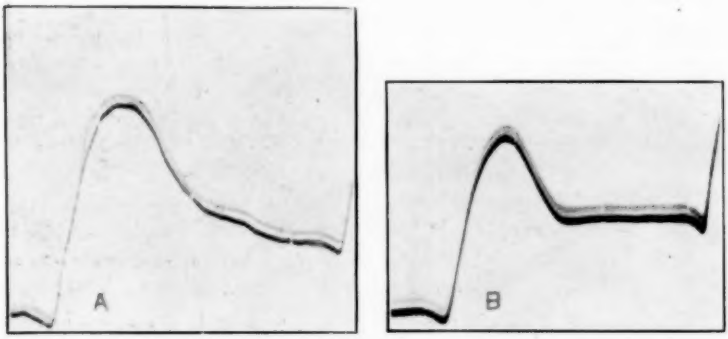

Fig. 6.—Examples of the deformity produced in the collapsing pulse by high pressures in the constricting cuff outside of the plethysmograph. In A a pressure of 90 mm. Hg was applied and in B a pressure of 118 mm. From the same patient as Figure 5. The average rate of flow in the arm was not diminished in spite of the deformity of the curve produced by the high pressure.

From our tracings, therefore, we conclude that in aortic insufficiency there is a marked back-flow in the brachial artery during the diastolic portion of the pulse cycle. This back-flow in the brachial artery is not dependent on gravity, for it has been obtained even when the arm is lowered below the general level of the body. Furthermore, this back-flow is not limited to the brachial artery, for in these patients a diastolic murmur can be produced in the femoral artery by a proper degree of compression. This phenomenon, Duroziez's sign, is difficult to explain in any other way than by assuming a back-flow in the femoral artery during diastole.

One might attempt to explain the very marked diastolic back-flows frequently seen in the brachial arteries of patients with the collapsing

pulse by assuming an extremely free flow of blood through the internal organs. Yet a similar degree of diastolic back-flow has not been encountered in any other condition, whereas it is the rule in the collapsing pulses of aortic insufficiency. It seems to us quite certain, therefore, that the marked diastolic back-flow is due to the regurgitant leak through the aortic valves and that when marked it is pathognomonic of this valve lesion.

PERIPHERAL REFLECTION IN AORTIC INSUFFICIENCY

There remains for consideration the well-marked back-flow in the brachial artery which extends from the end of the primary wave to the beginning of the dicrotic wave. If we assume that the latter corresponds approximately to the closure of the semilunar valves, then, as Stewart has pointed out, it is not possible to explain this back-flow as being due directly to the regurgitation of blood into the ventricle. It seems to us evident that this systolic back-flow in aortic insufficiency is in every way comparable to the similar back-flow of the nitroglycerin pulse and that like the latter, it depends on an elastic reflection of the primary pulse wave from the periphery. Indeed, the conditions in aortic insufficiency are peculiarly favorable for such a reflection, for a large quantity of blood is expelled by the ventricle into a vascular system already relaxed by the regurgitant leak. The large pulse wave travels through the relaxed arteries to the obstruction encountered in the finer arterioles and capillaries, and it may even enter the latter producing the capillary pulse. Our flow tracings, however, show that but a small proportion of the wave is allowed to pass the capillaries. The major portion distends the large arteries. A part is immediately thrown back by their elastic recoil, while another part is often returned toward the heart during diastole.

CONCLUSIONS

1. The brachial pulse flow in typical cases of the collapsing pulse of aortic insufficiency is characterized by (1) the large size of the primary wave, (2) by the common occurrence of a rapid back-flow during diastole and (3) by a marked reflection of the primary pulse wave.

2. The large primary wave depends in part on the unusual amount of blood expelled by the left ventricle during systole and in part on the relaxed condition of the arterial tree.

3. The back-flow during diastole is due mainly to the regurgitation into the left ventricle.

4. The back-flow during the latter portion of systole is due to an unusually marked reflection of the large primary pulse wave, produced by an elastic recoil of the peripheral arteries.

THE EFFECT OF TEMPORARY OCCLUSION OF RENAL CIRCULATION ON RENAL FUNCTION*

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Of great importance in relation to the surgery of the kidney is the question concerning the length of time that the renal vessels may be clamped without permanent injury to renal function or renal tissue. The question is dealt with here experimentally. The renal vessels in rabbits and dogs have been clamped for varying periods of time, and subsequently renal function has been tested by the excretion of phenolsulphonephthalein, lactose, potassium iodid, salt and water. The presence or absence of albumin and casts in the urine has been observed. The results of such functional studies have been controlled by histological examination of the kidneys.

The effect of obstruction to the renal circulation on the urinary picture and the histology of the kidney has already been investigated. Robinson[1] showed that tying of the renal vein resulted in the appearance of albumin, blood, or both, in the urine, and in enlargement of the kidney. This has been confirmed by other observers. Paneth,[2] Munk[3] and de Souza[4] have shown that following ligation of the renal vein, the urine is small in amount. Ludwig[5] found that clamping the renal vessels for a period of minutes frequently interfered with the secretion of urine for hours. Heidenhain[6] found that on freeing the vessels after a temporary clamping the secretion of urine did not begin at once. Litten[7] observed after clamping both renal vessels for periods up to two hours, that the kidney became enlarged and congested. If the renal artery alone was tied for one-half or one hour, albumin and casts appeared in the urine. Pathologically, the tubules were affected as shown by their

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* For review of literature concerning partial or complete obstruction to renal circulation see our previous paper. Rowntree, Fitz and Geraghty: Studies of Renal Function in Experimental Chronic Passive Congestion, THE ARCHIVES INT. MED., 1913, xi, 121.

1. Robinson: Med. Chir. Tr., 1843, xxvi, 51.
2. Paneth: Pflüger's Arch., 1886, xxxix, 515.
3. Munk: Berl. klin. Wchnschr., 1864, i, 333.
4. De Souza: Jour. Physiol., 1900, xxvi, 139.
5. Ludwig Physiologie, 1856, ii, 416.
6. Heidenhain: Hermann's Handb. d. Physiol., 1883, v, Teil 1, 321.
7. Litten: Ztschr. f. klin. Med., 1880, p. 131.

inability to stain with indigo carmin. But such changes were temporary. If the artery was clamped for two hours, permanent injury occurred, evidenced by tubular necrosis with cast production, cellular infiltration, resulting in circumscribed scar formation with calcification.

Chirié and Mayer[8] after temporary clamping of both renal veins in dogs, noted, in four out of seven cases, death with convulsions. Carrel[9] was unable to confirm this observation.

Eisendrath and Strauss[10] have studied the pathological changes in the kidneys of rabbits after compressing the renal vessels for from fifteen to ninety minutes. According to their results, temporary compression — half an hour or less — caused slight damage. If longer, marked permanent lesions occurred in the parenchyma, as evidenced by interstitial cellular infiltration, coagulation necrosis of tubular epithelium, and later by the deposition of calcium in and about the destroyed epithelium.

Thus it has been shown that clamping of the renal vessels for periods up to an hour may produce temporary anuria, albuminuria and cylindruria. If interference with the renal circulation is maintained sufficiently long, permanent interstitial increase in tissue occurs in the kidney. Except for the amount of urine excreted and the presence in it of albumin or casts, no systematic study of renal function under such conditions has been made.

AUTHORS' EXPERIMENTS

In our experiments rabbits and dogs were etherized and the abdomen opened aseptically in the median line. The left kidney was exposed, and by careful blunt dissection the vessels were freed from the surrounding tissues. The artery and vein were raised on an artery forceps with as little stretching as possible and a small rubber protected bull-dog clamp placed around them. In each experiment it was noted that on the distal side of the forceps the artery did not pulsate, while the vein at once became distended; on the proximal side the vein was collapsed and the artery pulsated normally. No attempt was made to prevent the capsular circulation. After the left renal vessels were clamped, the right renal vessels were ligated. The right kidney was removed and weighed. In a few animals, for control, the right kidney was left untouched.

While the clamp was in place, the abdomen was closed and covered with a warm saline pad and towel. To try to obviate the factor of shock, the body heat was maintained. The animals were anesthetized as lightly as possible. After the desired interval of time had elapsed, the clamp was removed, the macroscopic appearance of the kidney noted, and

8. Chirié and Mayer: Compt. rend. Soc. de biol., 1907, i, 598.
9. Carrel: Compt. rend. d. Soc. de biol., 1909, i, 527.
10. Eisendrath and Strauss: Jour. Am. Med. Assn., 1910, lv, 2286; see also Guthrie: The Archives Int. Med., 1910, v, 232.

the peritoneum and abdomen sewn up. The bladder was then emptied and the animals placed in metabolism cages. The clamp was applied in series of animals for ten, twenty, thirty, forty and sixty minutes. Repeated studies of renal function were made. Finally, the animals were killed, submitted to autopsy and the kidneys examined histologically after formaldehyd fixation, celloidin imbedding and hematoxylin and eosin staining.

TECHNIC OF TESTS OF RENAL FUNCTION

A short description of the various tests used for renal function, with their technic, is given.

The phthalein test was made according to the original technic of Rowntree and Geraghty.[11] One cubic centimeter of a phenolsulphonephthalein solution containing accurately 6 milligrams was injected aseptically into the leg muscles of the rabbits or lumbar muscles of the dogs, which were then placed in metabolism cages. The bladders were expressed or catheterized at the end of an hour and ten minutes, and the total urine for this time collected. The urine was made distinctly alkaline, diluted to 1 liter, and the amount of drug determined by the use of Rowntree and Geraghty's[12] modification of the Autenrieth-Königsberger colorimeter. In control animals it has been determined that the normal output in this time is 60 per cent. or more.

The lactose, potassium iodid, salt and water tests which have received a thorough study at the hands of Schlayer[13] and his co-workers and which we have used in relation to the renal function in experimental and clinical nephritides, were applied to this study.

From our previous work we feel that the mechanism of the excretion of lactose differs essentially from that of phthalein, salt and iodid. Throughout this investigation we have used it as an index of the condition of the vascular function[14] of the kidney, admitting that we need much more information concerning the manner and significance of its excretion.

The technic for the lactose test in rabbits has been identical with Schlayer's. One gram of lactose dissolved in 10 c.c. of distilled water was injected into the ear vein. The animals were placed in metabolism cages, the bladder expressed at the end of four hours and every hour thereafter up to eight hours. In dogs, according to our previous technic, 2 gm. were dissolved in 20 c.c. of water, and injected into the lumbar muscles.

Since the time necessary for total elimination has been considered by Schlayer of greater importance than the absolute amount recovered, and since our previous observations with lactose excretion agree with this, we have observed this exclusively. The presence of lactose in the urine was determined by Nylander's test, using similar amounts of urine, reagent and length of boiling time.

In rabbits under these conditions, the time necessary for the complete elimination of lactose is normally six hours or less and in dogs from four to six hours.

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11. Rowntree and Geraghty: Jour. Phar. and Exper. Therap., 1910, i, 579.

12. Rowntree and Geraghty: THE ARCHIVES INT. MED., 1912, ix, 284.

13. Schlayer: Deutsch. Arch. f. klin. Med., 1911, cii, 311; Schlayer and Takayasu: Deutsch. Arch. f. klin. Med., 1891, xcviii, 17; 1910, ci, 333.

14. For a review of the literature on this test and the others described below, see our previous paper. We have shown in a previous publication, however, that lactose is not excreted solely by the glomeruli.

According to the studies of Schlayer, potassium iodid is excreted by the tubules of the kidney, and on it he has placed most dependence in determining tubular functional capacity. In these studies 1 c.c. of a 2.5 per cent. solution has been administered intravenously to rabbits. For this the normal elimination time is twenty-four hours. In dogs, 0.5 gm. has been administered by mouth, which normally is excreted within sixty hours. The presence of the drug in the urine has been determined by Sandow's[15] test.

The excretion of salt following its administration in amounts greatly in excess of that ordinarily taken with the food is accomplished by the tubules, according to Schlayer. Normally, a large amount of salt is excreted by one of two methods. If it is given without extra water, it is almost entirely excreted within twenty-four hours without diuresis by increased salt concentration in the urine; if given with an excess of water, it is excreted partially through increased concentration in the urine and partially through diuresis.

Where vascular injury to the kidney exists, the simple administration of salt may be followed by a marked diuresis, all of the salt being excreted in twenty-four hours without its percentage content in the urine being at all increased. This is usually associated with a somewhat low and fixed specific gravity and the syndrome is spoken of as "vascular hypothenuria." Here the inability to concentrate is not due to any incapacity of the tubules to excrete salt, but to hypersensitive vessels which respond to the salt administration with a diuresis. In more severe vascular injury the vessels do not act in the same way, oliguria characterizing the urinary picture. In severe tubular destruction, a urine of fixed low specific gravity is obtained, the quantity of which is not materially affected by the administration of extra amounts of salt, and the salt content of which is not augmented by administration of extra amounts of salt because of the inability of the tubules to excrete it. Such a condition is known as "tubular hyposthenuria."

In these studies gm. 1.50 were given by stomach-tube to the rabbits, and gm. 3 to dogs. The urine for the following twenty-four hours was collected, and the salt concentration and absolute excretion estimated by the Lütke-Martius[16] method.

Finally, in order to make conditions as constant as possible, the rabbits were given 100 c.c. of water daily by stomach-tube. In addition,

15. Sandow's method consists in adding 1 c.c. of 2 per cent. sodium nitrite solution and 1 c.c. of 10 per cent. H_2SO_4 to from 10 to 30 c.c. of urine, followed by the addition of a small amount of chloroform. This is shaken together and allowed to separate into layers, the presence of the iodid being indicated by a purplish red or violet color in the chloroform.

16. Sahli: Lehrbuch der klinischen untersuchungs, Methoden, Franz Deuticke, Leipzig and Wien, 1899, p. 421.

they were allowed to eat green vegetables *ad libitum*. This produced a constant polyuria. Hence the amounts of urine were not followed from day to day. The dogs were allowed 300 c.c. of water daily, and were fed on meat when able to retain liquid or solid food. The lactose, phthalein and iodid tests were given synchronously since their excretion, and quantitative determination do not interfere with each other. In dogs, lactose and phthalein were given at the same time, but into the muscles of opposite sides.

Single catheter or expressed specimens of urine were taken from day to day. Albumin was tested by the nitric acid method. The sediment of centrifuged urine was examined microscopically for blood or casts.

As previously, in these studies we have considered the excretion of lactose as an index of vascular functional capacity; that of phthalein as an index of total renal function (though predominantly tubular), and the excretion of salt and iodid as an index of tubular functional capacity.

The results of these studies are shown in the accompanying protocols.[17]

PROTOCOLS OF EXPERIMENTS

I. Animals with one kidney removed, renal circulation clamped for 10 minutes.

RABBIT 1.—Body weight 1,650 gm. Weight of removed kidney 4.9 gm. Weight of remaining kidney at death 6.5 gm.

Sulphonephthalein excretion second day after operation 37 per cent.
Sulphonephthalein excretion third day after operation 62 per cent.
Sulphonephthalein excretion fifth day after operation 53 per cent.
Sulphonephthalein excretion seventh day after operation 58 per cent.
Lactose excretion first day after operation 6-7 hours.
Lactose excretion fourth day after operation 6-7 hours.
Iodid excretion first day after operation 36 hours +.
Iodid excretion fifth day after operation 36 hours +.
Salt excretion third day after operation 1.00 per cent. (1.20 gm.)
Salt excretion sixth day after operation 1.30 per cent. (1.00 gm.)

Albumin present for four days. Rare hyaline casts found until the seventh day. Animal killed on the eighth day.

RABBIT 2.—Weight 1,250 gm. Weight of removed kidney 4.6 gm. Weight of remaining kidney at death 4.5 gm.

Sulphonephthalein excretion second day after operation 65 per cent.
Sulphonephthalein excretion fourth day after operation 65 per cent.
Sulphonephthalein excretion fifth day after operation 62 per cent.
Sulphonephthalein excretion seventh day after operation 65 per cent.
Lactose excretion first day after operation 6 hours.
Lactose excretion fourth day after operation 7½ hours.
Lactose excretion seventh day after operation 5 hours.
Iodid excretion first day after operation 30 hours.
Iodid excretion fourth day after operation 24 hours.
Iodid excretion seventh day after operation 36 hours.

17. In addition seven rabbits were used, one with circulation clamped for ten minutes, three for thirty minutes, and two for forty minutes. All died within sixteen hours of operation. Realizing the individual variation in susceptibility and vitality of rabbits, it seemed fair to exclude these animals from their appropriate tables, especially as all had been deeply anesthetized, all but one had made poor ether recoveries, and one rabbit at operation had suffered from a severe hemorrhage due to trauma of the liver.

Salt excretion third day after operation 1.25 per cent. (1.00 gm.)
Salt excretion sixth day after operation 1.3 per cent. (1.3 gm.)

Albumin present for six days. Sediment negative throughout the experiment. Animal found dead on the eighth day. No cause found.

RABBIT 3.—Weight 2,250 gm. Weight of removed kidney 6 gm. (approx.). Weight of remaining kidney at death 8 gm.

Sulphonephthalein excretion first day after operation 9 per cent.
Sulphonephthalein excretion second day after operation 24 per cent.
Sulphonephthalein excretion third day after operation 35 per cent.
Sulphonephthalein excretion sixth day after operation 62 per cent.
Sulphonephthalein excretion eighth day after operation, 80 per cent.
Lactose excretion first day after operation 8 hours +.
Lactose excretion sixth day after operation 6 hours.
Lactose excretion thirteenth day after operation 5 hours.
Iodid excretion first day after operation 30 hours.
Iodid excretion sixth day after operation 34 hours.
Salt excretion third day after operation .72 per cent. (1.35 gm.)
Salt excretion eighth day after operation .56 per cent. (1.40 gm.)

Albumin present for three days. Sediment showed rare hyaline casts on the thirteenth day. Hematuria for three days. The animal was killed on the fourteenth day.

II. Animals with one kidney removed. Renal circulation clamped for twenty minutes.

RABBIT 1.—Weight 1,800 gm. Weight of removed kidney 6.6 gm. Weight of remaining kidney at death 5.5 gm.

Sulphonephthalein excretion first day after operation 32 per cent.
Sulphonephthalein excretion third day after operation 43 per cent.
Sulphonephthalein excretion fifth day after operation 42 per cent.
Sulphonephthalein excretion thirteenth day after operation 48 per cent.
Sulphonephthalein excretion twentieth day after operation 37 per cent.
Lactose excretion first day after operation 7½ hours.
Lactose excretion fifth day after operation 6 hours.
Lactose excretion fourteenth day after operation 5 hours.
Lactose excretion twentieth day after operation 7 hours +.
Iodid excretion first day after operation 24 hours.
Iodid excretion fourteenth day after operation 24 hours.
Iodid excretion twentieth day after operation 24 hours.
Salt excretion third day after operation 1.00 per cent. (1.1 gm.)
Salt excretion seventh day after operation .8 per cent. (2.00 gm.)
Salt excretion sixteenth day after operation .7 per cent. (1.6 gm.)

Albumin present for seven days. Few hyaline casts found until the seventh day. Hematuria for five days. Animal found dead on the twenty-seventh day. Histologically the kidney not remarkable.

RABBIT 2.—Weight 1,720 gm. Weight of removed kidney 6.4 gm. Weight of remaining kidney at death 6.5 gm.

Sulphonephthalein excretion first day after operation 27 per cent.
Sulphonephthalein excretion fifth day after operation 32 per cent.
Sulphonephthalien excretion sixth day after operation 60 per cent.
Sulphonephthalien excretion fourteenth day after operation 58 per cent.
Sulphonephthalien excretion twentieth day after operation 58 per cent.
Sulphonephthalein excretion twenty-eighth day after operation 64 per cent.
Lactose excretion first day after death 7½ hours.
Lactose excretion fifth day after operation 7 hours.
Lactose excretion fourteenth day after operation 5 hours.
Lactose excretion twenty-eighth day after operation 5 hours.
Iodid excretion first day after operation 24 hours.
Iodid excretion fifth day after operation 30 hours.
Iodid excretion fourteenth day after operation 24 hours.
Iodid excretion twenty-eighth day after operation 24 hours.
Salt excretion third day after operation .6 per cent. (1.1 gm.).
Salt excretion seventh day after operation 1.2 per cent. (1.8 gm.).

Salt excretion sixteenth day after operation .53 per cent. (1.3 gm.).
Salt excretion twenty-eighth day after operation .46 per cent. (2.00 gm.).

Albumin present for three days. Hematuria for four days. No casts found after the fifth day. Animal killed the twenty-eighth day.

RABBIT 3.—Weight 2,020 gm. Weight of removed kidney 6.5 gm. Weight of remaining kidney at death 7.00 gm.

Sulphonephthalein excretion first day after operation 55 per cent.
Sulphonephthalein excretion third day after operation 35 per cent.
Sulphonephthalein excretion fifth day after operation 65 per cent.
Sulphonephthalein excretion fifteenth day after operation 64 per cent.
Sulphonephthalein excretion twenty-eighth day after operation 70 per cent.
Lactose excretion first day after operation 7½ hours.
Lactose excretion fifth day after operation 4 hours.
Lactose excretion fifteenth day after operation 4 hours.
Lactose excretion twenty-third day after operation 6 hours.
Iodid excretion first day after operation 24 hours.
Iodid excretion fifth day after operation 24 hours.
Iodid excretion twenty-third day after operation 24 hours.
Salt excretion third day after operation 1.00 per cent. (1.1 gm.).
Salt excretion twelfth day after operation .8 per cent. (2.4 gm.).

Albumin present for three days. Hematuria for one day. No casts found after the sixth day. Animal killed the twenty-eighth day.

DOG 1.—Weight 5 kilos. Weight of the removed kidney 20 gm.

Sulphonephthalein excretion first day after operation 40 per cent.
Sulphonephthalein excretion second day after operation 60 per cent.
Sulphonephthalein excretion fifth day after operation 50 per cent.
Sulphonephthalein excretion ninth day after operation 68 per cent.
Sulphonephthalein excretion twelfth day after operation 60 per cent.
Lactose excretion first day after operation 8 hours +.
Lactose excretion fifth day after operation 8 hours.
Lactose excretion ninth day after operation 5 hours.
Iodid excretion second day after operation 48 hours.
Iodid excretion twelfth day after operation 48 hours.
Salt excretion second day after operation .85 per cent. (3.00 gm.).

Albumin present for one day. No casts found after the second day. The animal was allowed to live, since he had made an apparently normal recovery.

III.—Animals with one kidney removed; renal circulation clamped for thirty minutes.

RABBIT 1.—Weight 1,700 gm. Weight of removed kidney 5 gm. (Approx.) Weight of remaining kidney after death 6.5 gm.

Sulphonephthalein excretion first day after operation 46 per cent.
Sulphonephthalein excretion second day after operation 40 per cent.
Sulphonephthalein excretion fourth day after operation 60 per cent.
Sulphonephthalein excretion thirteenth day after operation 67 per cent.
Sulphonephthalein excretion twenty-eighth day after operation 72 per cent.
Sulphonephthalein excretion thirty-first day after operation 60 per cent.
Lactose excretion first day after operation 6½ hours.
Lactose excretion fourth day after operation 5 hours.
Lactose excretion thirteenth day after operation 5 hours.
Lactose excretion twenty-eighth day after operation 5 hours.
Iodid excretion first day after operation 36 hours.
Iodid excretion fourth day after operation 30 hours.
Iodid excretion thirteenth day after operation 24 hours.
Salt excretion third day after operation 1.3 per cent. (1.7 gm.).
Salt excretion sixth day after operation .8 per cent. (1.4 gm.).
Salt excretion thirty-first day after operation .9 per cent. (1.4 gm.).

Albuminuria for four days. No casts found after the thirteenth day. Animal killed on the thirty-first day.

RABBIT 2.—Weight 2,400 gm. Weight of removed kidney 7.6 gm. Weight of remaining kidney after death 11.5 gm.

Sulphonephthalein excretion first day after operation 36 per cent.
Sulphonephthalein excretion second day after operation 40 per cent.
Sulphonephthalein excretion fourth day after operation 70 per cent.
Sulphonephthalein excretion fifteenth day after operation 62 per cent.
Lactose excretion first day after operation 7 hours +.
Lactose excretion fourth day after operation 5 hours.
Lactose excretion fifteenth day after operation 5 hours.
Iodid excretion fifteenth day after operation 24 hours.
Salt excretion first day after operation .7 per cent. (1.6 gm.).
Salt excretion fifteenth day after operation .6 per cent. 2.4 gm.).

Albumin present for one day. Hematuria for two days. No casts found after the fourth day. Animal killed the fifteenth day.

RABBIT 3.—Weight 2,600 gm. Weight of removed kidney 9.6 gm. Weight of remaining kidney after death 8 gm.

Sulphonephthalein excretion second day after operation 55 per cent.
Sulphonephthalein excretion fourth day after operation 70 per cent.
Sulphonephthalein excretion fifteenth day after operation 80 per cent.
Sulphonephthalein excretion twentieth day after operation 74 per cent.
Lactose excretion first day after operation 7 hours.
Lactose excretion fourth day after operation 5 hours.
Lactose excretion fourteenth day after operation 7 hours.
Lactose excretion twentieth day after operation 5 hours.
Iodid excretion first day after operation 24 hours.
Iodid excretion fourth day after operation 24 hours.
Iodid excretion fifteenth day after operation 24 hours.
Salt excretion third day after operation 1.1 per cent. (2.5 gm.).
Salt excretion fourteenth day after operation .52 per cent. (2.1 gm.).
Salt excretion twentieth day after operation .65 per cent. (1.9 gm.).

Albumin present for one day. Hematuria for one day. No casts seen after the third day. Animal killed the thirtieth day.

DOG 1.—Weight 7 kilos. Weight of removed kidney 24 gm.

Sulphonephthalein excretion second day after operation 36 per cent.
Sulphonephthalein excretion third day after operation 52 per cent.
Sulphonephthalein excretion fifth day after operation 55 per cent.
Lactose excretion third day after operation 8 hours.
Iodid excretion second day after operation 48 hours.
Salt excretion third day after operation 1 per cent. (1.6 gm.).

Albumin for two days. Casts found on the fifth day. Animal made an uneventful recovery and allowed to live.

IV.—Animal with one kidney removed; renal circulation clamped for forty minutes.

RABBIT 1.—Weight 2,070 gm. Weight of removed kidney 5.7 gm. Weight of remaining kidney after death 7.2 gm.

Sulphonephthalein excretion first day after operation 24 per cent.
Sulphonephthalein excretion second day after operation 46 per cent.
Sulphonephthalein excretion third day after operation 50 per cent.
Sulphonephthalein excretion fourth day after operation 62 per cent.
Sulphonephthalein excretion eighth day after operation 70 per cent.
Lactose excretion second day after operation 6 hours.
Lactose excretion eighth day after operation 6 hours.
Lactose excretion twelfth day after operation 5 hours.
Iodid excretion first day after operation 48 hours.
Salt excretion fourth day after operation 1 per cent. (1.5 gm.).
Salt excretion tenth day after operation .7 per cent. (1.2 gm.).

Albuminuria for eight days. Hematuria for one day. No casts found after the fourth day. Animal killed on the twelfth day on account of general infection.

RABBIT 2.—Weight 1,600 gm. Weight of removed kidney 4.6 gm. Weight of remaining kidney after death 6.5 gm.

Sulphonephthalein excretion first day after operation 46 per cent.
Sulphonephthalein excretion second day after operation 47 per cent.

Sulphonephthalein excretion third day after operation 56 per cent.
Lactose excretion first day after operation 8 hours +.
Lactose excretion third day after operation 7 hours +.
Iodid excretion first day after operation 30 hours.
Iodid excretion third day after operation 24 hours.
Salt excretion third day after operation 1 per cent. (1.2 gm.)

Albumin and casts present until death. Animal killed on the fourth day on account of general infection.

RABBIT 3.—Weight 2,200 gm. Weight of removed kidney 6.00 gm. Weight of remaining kidney after death 9.00 gm.

Sulphonephthalein excretion first day after operation 45 per cent.
Sulphonephthalein excretion second day after operation 54 per cent.
Sulphonephthalein excretion fourth day after operation 60 per cent.
Sulphonephthalein excretion fifth day after operation 70 per cent.
Lactose excretion first day after operation 8 hours.
Lactose excretion third day after operation 5 hours.
Lactose excretion fifth day after operation 4 hours.
Iodid excretion first day after operation 30 hours.
Iodid excretion third day after operation 24 hours.
Salt excretion second day after operation .9 per cent. (1.00 gm.)
Salt excretion fifth day after operation .9 per cent. (2.5 gm.)

Albumin present for three days. Hematuria for one day. No casts seen after the fourth day. Animal killed on the sixth day.

RABBIT 4.—Weight 1,770 gm. Weight of removed kidney 5.00 gm. Weight of remaining kidney after death 7.00 gm.

Sulphonephthalein excretion first day after operation 15 per cent.
Sulphonephthalein excretion second day after operation 14 per cent.
Sulphonephthalein excretion third day after operation 8 per cent.
Lactose excretion first day after operation 8 hours +.
Lactose excretion third day after operation 6 hours.
Iodid excretion first day after operation 30 per cent.
Salt excretion second day after operation 1.00 per cent. (.7 gm.)

No albumin found after the second day. Hematuria for one day. Casts seen on the third day. Animal found dead on the fourth day with acute peritonitis.

RABBIT 5.—Weight 2,320 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 10 gm.

Sulphonephthalein excretion first day after operation 47 per cent.
Sulphonephthalein excretion fourth day after operation 62 per cent.
Sulphonephthalein excretion twelfth day after operation 67 per cent.
Sulphonephthalein excretion fourteenth day after operation 70 per cent.
Lactose excretion second day after operation 6 hours.
Lactose excretion fourth day after operation 6 hours.
Lactose excretion thirteenth day after operation 5 hours.
Iodid excretion second day after operation 24 hours.
Salt excretion second day after operation .7 per cent. (1.3 gm.)
Salt excretion thirteenth day after operation .9 per cent. (2.5 gm.)

Albuminuria for two days. Casts present for four days. Animal killed on the fifteenth day.

DOG 1.—Weight 6 kilos. Weight of removed kidney 33 gm. Weight of remaining kidney after death 50 gm.

Sulphonephthalein excretion first day after operation traces.
Sulphonephthalein excretion second day after operation traces.
Sulphonephthalein excretion third day after operation traces.
Lactose excretion first day after operation 8 hours +.
Lactose excretion second day after operation 8 hours.
Salt and iodid not given. Albumin, blood and casts present until death.

Animal killed on the fourth day on account of extreme toxemia.

DOG 2.—Weight 10 kilos. Weight of removed kidney 30 gm.

Sulphonephthalein excretion first day after operation 20 per cent.
Sulphonephthalein excretion third day after operation 30 per cent.

Sulphonephthalein excretion fourth day after operation 64 per cent.
Sulphonephthalein excretion seventh day after operation 68 per cent.
Lactose excretion first day after operation 8 hours +.
Lactose excretion fourth day after operation 8 hours +.
Lactose excretion seventh day after operation 6 hours.
Iodid excretion second day after operation 48 hours.
Salt excretion second day after operation .4 per cent. (.80 gm.)

Albumin present for four days. No casts seen after the fourth day. Animal made an uneventful recovery and allowed to live.

V.—Animals with one kidney removed; renal circulation clamped for one hour.

RABBIT 1.—Weight 1,750 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 7.5 gm.

Sulphonephthalein excretion first day after operation 32 per cent.
Sulphonephthalein excretion third day after operation 35 per cent.
Lactose excretion second day after operation 8 hours +.
Iodid excretion second day after operation 2 hours +.
Salt excretion second day after operation .6 per cent. (.5 gm.)

Albumin and casts found on the third day. Hematuria for one day. Animal found dead on the fourth day. Autopsy showed acute peritonitis.

RABBIT 2.—Weight 2,000 gm. Weight of removed kidney 5.2 gm. Weight of remaining kidney after death 6.5 gm.

Sulphonephthalein excretion first day after operation 7 per cent.
Lactose excretion second day after operation 8 hours +.
Salt excretion second day after operation .7 per cent. (.8 gm.)

Albumin, casts and blood in the urine on the third day. Animal found dead.

RABBIT 3.—Weight 1,800 gm. Weight of removed kidney 6.00 gm. Weight of remaining kidney after death 9.00 gm. Animal died anuric in seventy-two hours.

RABBIT 4.—Weight 1,700 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 9.5 gm.

Sulphonephthalein excretion first day after operation 9 per cent.
Sulphonephthalein excretion second day after operation 12 per cent.
Sulphonephthalein excretion third day after operation 20 per cent.
Sulphonephthalein excretion sixth day after operation 8 per cent.
Lactose excretion first day after operation 8 hours +.
Lactose excretion third day after operation 8 hours +.
Iodid excretion first day after operation 48 hours.
Salt excretion third day after operation .8 per cent. (2.1 gm.)

Albumin and casts present until death. Animal found dead a few days after last note. Phthalein excreted in traces, lactose markedly delayed.

RABBIT 5.—Weight 1,400 gm. Weight of removed kidney 4 gm. Weight of remaining kidney after death 9.5 gm.

Sulphonephthalein excretion first day after operation traces.
Sulphonephthalein excretion second day after operation traces.
Sulphonephthalein excretion third day after operation traces.
Lactose excretion first day after operation none recovered.

Albumin, blood and casts present until death on the fourth day. During the last twenty-four hours passed 10 c.c. of urine.

VI.—Animals in which the renal circulation has been clamped in one kidney for an hour, the other kidney being left untouched.

RABBIT 1.—Weight 1,650 gm. Weight of unclamped kidney at death 5.00 gm. Weight of clamped kidney at death 5.00 gm.

Sulphonephthalein excretion first day after operation 30 per cent.
Sulphonephthalein excretion second day after operation 52 per cent.
Sulphonephthalein excretion sixth day after operation 56 per cent.
Sulphonephthalein excretion eighth day after operation 75 per cent.
Sulphonephthalein excretion eighteenth day after operation 60 per cent.

Sulphonephthalein excretion twenty-second day after operation 70 per cent.
Sulphonephthalein excretion twenty-fifth day after operation 76 per cent.
Lactose excretion first day after operation 7 hours.
Lactose excretion fourth day after operation 6 hours.
Lactose excretion eighth day after operation 5 hours.
Lactose excretion fifteenth day after operation 6 hours.
Lactose excretion twenty-fifth day after operation 5 hours.
Iodid excretion first day after operation 24 hours.
Iodid excretion eighth day after operation 24 hours.
Iodid excretion twenty-second day after operation 24 hours.
Salt excretion sixth day after operation .5 per cent. (1.1 gm.).
Salt excretion twenty-fifth day after operation .76 per cent. (1.2 gm.)

Albumin present for two days. No casts found after the sixth day. Animal killed on the twenty-sixth day.

RABBIT 2.—Weight 2,200 gm. Weight of unclamped kidney at death 7.5 gm. (approx.). Weight of clamped kidney at death 7.5 gm.

Sulphonephthalein excretion first day after operation 59 per cent.
Sulphonephthalein excretion fifth day after operation 74 per cent.
Sulphonephthalein excretion seventeenth day after operation 78 per cent.
Lactose excretion third day after operation 6 hours.
Lactose excretion tenth day after operation 7 hours +.
Lactose excretion eighteenth day after operation 6 hours.
Iodid excretion third day after operation 24 hours.
Iodid excretion eighteenth day after operation 24 hours.
Salt excretion eighteenth day after operation .3 per cent. (1.20 gm.)

Albumin present for one day. No casts seen after the fifth day. Animal killed on the nineteenth day.

RABBIT 3.—Weight 1,700 gm.

Sulphonephthalein excretion first day after operation 50 per cent.
Sulphonephthalein excretion third day after operation 58 per cent.
Sulphonephthalein excretion seventh day after operation 70 per cent.
Lactose excretion third day after operation 6 hours.
Lactose excretion seventh day after operation 5 hours.
Iodid excretion first day after operation 24 hours.
Iodid excretion seventh day after operation 24 hours.
Salt excretion fifth day after operation .7 per cent. (1.4 gm.)

Albumin present for one day. Casts found on the seventh day. Animal found dead on the twelfth day. No cause found.

VII.—Animals in which one kidney has been removed, the circulation of the other being left untouched.

RABBIT 1.—Weight 1,900 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 7.5 gm.

Sulphonephthalein excretion first day after operation 50 per cent.
Sulphonephthalein excretion second day after operation 65 per cent.
Sulphonephthalein excretion fifth day after operation 60 per cent.
Sulphonephthalein excretion ninth day after operation 70 per cent.
Lactose excretion first day after operation 5 hours.
Lactose excretion ninth day after operation 5 hours.
Lactose excretion sixteenth day after operation 6 hours.
Iodid excretion first day after operation 24 hours.
Iodid excretion sixteenth day after operation 24 hours.
Salt excretion third day after operation .5 per cent. (2.00 gm.)

Traces of albumin found for one day. Blood and casts found for one day. Animal killed on the twentieth day.

RABBIT 2.—Weight 2,200 gm. Weight of removed kidney 6 gm. Weight of remaining kidney after death 8.5 gm.

Sulphonephthalein excretion first day after operation 69 per cent.
Sulphonephthalein excretion second day after operation 70 per cent.
Sulphonephthalein excretion twelfth day after operation 75 per cent.
Sulphonephthalein excretion thirteenth day after operation 80 per cent.

Lactose excretion first day after operation 5 hours.
Lactose excretion thirteenth day after operation 5 hours.
Iodid excretion first day after operation 24 hours.
Iodid excretion thirteenth day after operation 24 hours.
Salt excretion third day after operation .3 per cent. (.7 gm.)
Salt excretion thirteenth day after operation .26 per cent. (1.1 gm.)
Albumin present for one day. No casts seen. Animal killed on the fourteenth day.

RABBIT 3.—Weight 1,850 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 7 gm.
Sulphonephthalein excretion first day after operation 25 per cent.
Sulphonephthalein excretion second day after operation 30 per cent.
Sulphonephthalein excretion fifth day after operation 55 per cent.
Sulphonephthalein excretion twentieth day after operation 70 per cent.
Lactose excretion first day after operation 5 hours.
Lactose excretion nineteenth day after operation 5 hours.
Iodid excretion first day after operation 24 hours.
Iodid excretion nineteenth day after operation 24 hours.
Salt excretion third day after operation .6 per cent.
Salt excretion nineteenth day after operation 1.1 per cent. (1.5 gm.)
Albumin present for one day. Hematuria for one day. Animal killed on the twenty-first day.

Clamping of the renal circulation up to forty minutes, in the majority of cases, produced a definite disturbance in renal function. Its intensity bore no relation to the length of time the vessels were clamped, nor was the vascular or tubular function chiefly affected (Protocols I to IV). This was shown by the presence of albumin and casts in the urine, by a diminished phthalein output of varying degree, and by a delayed lactose and iodid excretion. Salt was constantly well excreted. The animals recovering, regained nearly normal function within six days, showing that the disturbance was slight and temporary. One animal failed to return to a normal phthalein output. No explanation was found for this at autopsy. Two animals with circulation clamped for forty minutes died quickly with marked signs of renal insufficiency.

All the animals, however, with one kidney removed and the circulation of the remaining kidney clamped for an hour (Protocol V) died within eight days. There was evidence clinically as well as by these tests of extreme disturbance of function. An interesting proof of how important a part in renal surgery the unoperated kidney plays is seen in Protocol VI. In this series, though the circulation was clamped for an hour, with a normal kidney remaining, the function was but slightly and temporarily disturbed.

That the effect of nephrectomy alone did not produce the results obtained is seen from Protocol VII. In two animals the functional tests were unaffected. In the third the phthalein output was slightly reduced for three days. The other tests were normal.

Pathologically, similar changes were noted to those described by previous authors. The earliest changes, grossly, were marked congestion with scattered minute hemorrhages. As the length of time following

the operation increased, the kidneys hypertrophied, but otherwise appeared normal.

Microscopically first were seen hemorrhage, edema, destruction of cells, as evidenced by poor staining, the presence of hyaline-like casts in the tubules, and foci of leukocytes, endothelial phagocytes and mononuclear cells between the tubules and around the glomeruli. In time the kidney regained nearly normal appearance. In a few kidneys there were areas of increased interstitial tissue, and rarely thickening of a few glomerular capsules. It was not possible to find any definite relationship between the anatomical changes and the disturbance of function, except that in those kidneys clamped for an hour there was more edema and necrosis than in the others. In none of the kidneys examined was there evidence of any progressive lesion. The changes were all acute or healed.

From these experiments on animals it seems clear that renal circulation may be clamped for at least forty minutes without danger of marked permanent damage to the kidney either histologically or functionally. How far these results are applicable to human surgery is an open question. From the fact that these experiments were made under the most disadvantageous conditions possible, that is, with only one functionating kidney, it would seem fair to assume that in man with one kidney normal, the circulation of the other kidney could be clamped for a considerable length of time without harm.

CONCLUSIONS

1. In rabbits and dogs with one kidney removed, the circulation of the other kidney may be clamped for as long a time as forty minutes with recovery. If the renal circulation is clamped for a longer time, the animals die with signs of renal insufficiency.

2. In animals with the circulation clamped for not longer than forty minutes, temporary disturbance in renal function is produced as shown by the presence of albumin and casts in the urine, by a diminished phthalein output and by a delayed lactose and iodid excretion. Normal function is regained within six days.

3. Acute or healed pathological changes are found in kidneys so treated. The acute changes consist of edema, hemorrhage, necrosis and cellular infiltration. The healed changes consist in foci of connective tissue. No progressive lesion is found.

4. Except in the most extreme cases, there is no definite relation demonstrable by the functional tests used between the pathological and functional disturbances produced.

5. In rabbits with one normal kidney, the circulation of the other kidney may be clamped for at least an hour without permanent injury to the animal's general condition or renal function.

OBSERVATIONS ON THE PATHOLOGICAL CHANGES IN THE THYROID GLAND IN A CRETINISTIC VARIETY OF CHONDRODYSTROPHIA FOETALIS *

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That the lesion variously known as chondrodystrophia foetalis, achondroplasia, fetal rickets and the like, is of great antiquity is attested by the numerous instances in which the disease finds representation among pottery and statuary of the ancients (Parrot,[1] Pierre Marie,[2] Porak and Durante,[3] Regnault,[4] Charcot and Richie,[5] Rischbieth[6] and others). The models of the Egyptian gods, Ptah and Bes, to be found in the Louvre, the existing statuette in caricature of the Roman Emperor Caracalla, the figures of the gladiator dwarfs in the service of the Emperor Domitian, as well as the subjects of certain paintings, notably by Velasquez, among them that of Sebastiano de Moro, a dwarf of the Spanish Court, are all recognizable examples of fetal chondrodystrophy.

Historical interest attaches to the statement that, in the sixteenth century, Catherine de Medici revived the ancient custom of according gnome-like creatures a position in the social firmament of the Court, where they enjoyed unusual latitude of speech and action in the capacity of jesters, and emulating the Emperor Heliogabalus, she sought even further to gratify her perverted taste by celebrating marriages between dwarfs with the purpose of perpetuating a stunted race comparable to the ethnic dwarfs of fact and fable. A survival of the same perversion consists in exploiting the chondrodystrophic dwarf in the circus of to-day that his deformed body may be held up to gibe, ridicule or vulgar curiosity. On the other hand, numbers of characters distinguished by action or intellect are known or are reputed to have been the victims of fetal chondrodystrophy, among them the author of Æsop's Fables;

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1. Parrot: Bull. Soc. d'anthropolog. de Paris, Series 3, 1878, i, 296.
2. Marie, Pierre: Presse méd. 1900, iv, 12.
3. Porak and Durante: Nouvelle Incongraphie de la Salpêtrière, 1905, xviii, 481.
4. Regnault: Arch. gén. de méd. (new series), 1902, vii, 232.
5. Charcot and Richie: Des difformes et les malades dans l'ast, Paris, 1889, p. 12.
6. Rischbieth: Eugenics Laboratory Memoirs, xv, Parts vii and viii, Section xva, p. 355, London, 1912.

Philetas of Cos, tutor of Ptolemy Philadelphus and a poet and grammarian of renown; Licinius Calvus, a rhetorician; Atilla, King of the Huns; Wladislaus, called "Cubitalis," King of Poland, and noted for intelligence and military sagacity, and others of like account.

From an etiological standpoint chondrodystrophia foetalis is scarcely less obscure than it was in the beginning and of the several factors which have been invoked to explain its origin none appears to be worthy of serious consideration other than that the condition is always congenital, completing its development in the first few weeks of intra-uterine existence, that it preponderates in the female sex (Kassowitz), and that it sometimes presents familial characteristics. In the latter connection Rischbieth,[6] who has contributed an admirable paper to the eugenics of dwarfism, states that, while a chondrodystrophic parent usually begets normal children, heredity not uncommonly displays itself in the shape of a deformed progeny. The pathogenesis is no less conjectural for having been referred to various types of intoxication, including infections and placental and glandular perversions. In the latter connection Abels[7] quotes a suggestive instance in which a chondrodystrophic infant was born of a mother who, before and during pregnancy, had accustomed herself to enormous quantities of thyroid extract. Hofmeister,[8] on the contrary, states that extirpation of the thyroid gland from young rabbits is followed by cessation of growth in the bones and by changes which are comparable to those encountered in chondrodystrophia foetalis.

The first really adequate contribution to the pathology of chondrodystrophia foetalis was made in 1860 by Mueller,[9] who observed the disease in certain forms of cattle and identified the changes with those previously noted by Virchow[10] in the human skeleton. Mueller's observations have since been extended to include certain short-limbed domestic animals, more particularly the dachshund, bull dog, Pekinese spaniel, and Aberdeen terrier, but observation has shown that the likeness between them and the subjects of chondrodystrophia foetalis is fancied rather than real and that these animals merely typify combinations of different racial peculiarities brought about by artificial selection.

It was reserved for Kaufmann,[11] in 1892, to establish a classification based on anatomical and histological studies in human beings which showed that chondrodystrophia foetalis is referable to disturbances in the primordial cartilage cells manifested by derangement of the columnar

7. Abels: Kassowitz' Festschrift, Berlin, 1912, p. 1, quoting Cavazanni, Pediatrica, 1907.

8. Hofmeister: Quoted by Kaufmann, Spezielle path. Anat., 1911, ii, Part 6, p. 733.

9. Mueller: Wurzb. med. Zeit., 1860, i.

10. Virchow: Gesammte Abhandlung., 1852.

11. Kaufmann: Untersuchungen u. die sogenannte foetale Rachitis, 1892; Spezielle path. Anat., 1911, ii, Part 6, p. 733.

formation which normally precedes ossification. Depending on the extent of these cellular variations in cartilage, Kaufmann differentiated three anatomical types, all of which may be found in the same individual, although, as a rule, one of them is apt to dominate. In the first type — chondrodystrophia foetalis hypoplastica — proliferation of cartilage cells is inhibited, while the second variety — chondrodystrophia foetalis hyperplastica — is characterized by cartilaginous overgrowth. In both of these forms the intercellular substance is well preserved. In a third type — chondrodystrophia foetalis malacicca — the cartilage is soft, the intercellular substance is gelatinous and centers of ossification are rudimentary or absent. In addition to these cartilage changes endochondral ossification is prematurely suspended, while periosteal osteogenesis continues, so that several factors combine to interfere with or to modify the growth of certain bones and to produce a clinical picture which is at once characteristic and scarcely to be mistaken for that of any other known disease.

Individuals of the chondrodystrophic type, the majority of whom are still-born or die young, are deformed in certain bones which are conceived in cartilage and whose scheme of development is completed before the eleventh week of embryonal life;[12] that is to say, the osseous complements of the base of the skull and the pelvis, the ribs and the long bones of the extremities. Consequently the child at birth is greatly abbreviated in stature, the head is large, and the extremities, which may be crooked, are symmetrically shortened and thickened, varying between micromelia and phocomelia, and the cartilaginous termini are enlarged and nodular. The finger-tips, which normally reach to the level of the upper third of the thigh, rest at the crest of the ilium or shortly below it. The hands are broad and pudgy and the middle digit is shortened. The fingers are roughly conical in outline and separate in pairs, combining with the out-stretched thumb to form the *"main en trident"* of the French. The face is relatively small, the nose depressed at the root and the frontal eminences are exaggerated. These features unite with the large cranial vault to lend a pyriform outline to the face. The trunk is usually well formed and of normal size, although the abdomen frequently is protuberant. The skin, which develops out of proportion to the growth of the bones, is thrown into folds, especially over the joints and corresponding to the normal creases. It is often waxy in appearance and the subdermal tissues may be edematous or excessively infiltrated by fat. In those who attain adult life these characteristics are preserved, with the exception of certain modifications referable to the skin, but as development proceds the individual is endowed with muscular strength, sexual vigor and a degree of intelligence often noticeably in excess of the average. In addition to alterations in the bony skeleton, certain

12. Consult Mall: Am. Jour. Anat., 1905-6, v, 433.

numbers of chondrodystrophic individuals present external signs that are suggestive of cretinism; so much so, indeed, that some observers have proclaimed that chondrodystrophia foetalis and infantile myxedema are manifestations of the same fundamental disorder, or, at all events, that the two conditions are closely related. Kaufmann and his adherents deny such relationship. While admitting that there is a form of chondrodystrophia foetalis in which the physiognomy is cretinoid in type, they affirm that it is due almost entirely to premature synostosis of the os tribasilare and that it is an integral part of the chondrodystrophic process, arising independently of alterations in the thyroid gland. It is furthermore pointed out that chondrodystrophic individuals sometimes reach adult life, in which event, in contra-distinction to cretins, they are intelligent, sexually capable and possessed of muscular strength.

The relationship of chondrodystrophia foetalis to cretinism was originally insisted on by Mueller[9] and by Eberth,[13] both of whom pointed out that the so-called "bull dog calf" presents cretinoid appearances. Some years later Leblanc[14] noted the association of chondrodystrophia foetalis and myxedema in calves and attempted to implicate the thyroid gland as the causative factor in the production of both the skeletal and myxomatous changes. He based this conclusion on a single observation of "arrested development" in the thyroid! Histological examination was not made. It remained for Seligmann[15] to demonstrate tangible changes in the thyroid gland of the "bull dog calf" and to establish the coexistence, in cattle at least, of chondrodystrophia foetalis and congenital myxedema. Seligmann examined specimens of Dexter calves born in Kerry. In all of them the skin was thick and coarse and the subcutaneous tissues were myxomatous. The head was large and brachycephalic, the forehead bulging and the nose depressed. The tongue was large and protruded from the half-open mouth. All the limbs were extremely reduced in length and their bones were stunted and provided with thickened, deformed, softened cartilages. In every instance the thyroid was irregularly lobulated and, on microscopic examination, the alveolar architecture was scarcely recognizable and the cells were arranged in clumps or branching columns. Colloid was practically absent and the gland was abnormally well vascularized.

As to the influence of the thyroid in the production or modification of chondrodystrophia foetalis in human beings, we have been able to find very little in the literature. Kaufmann, Legry and Regnault,[16] and others have submitted the thyroid to histological examination with nega-

13. Eberth: Festschrift, Leipzig, 1878.
14. Leblanc: Compt. rend. Soc. de biol., 1902, liv, 88.
15. Seligmann: Trans. Path. Soc. London, 1904, lv, 1.
16. Legry and Regnault: Compt. rend. Soc. de biol., 1902, liv, 567.

tive results. In the same way Marie,[2] Cestan[17] and Christopher[18] have failed to note beneficial effects from the administration of thyroiodin and thyroid extract. Hektoen,[19] however, has recorded an instance of symmetrical micromelic dwarfism in a fairly intelligent man about 45 years of age, whose bony frame-work was the seat of a series of complex changes partaking of the nature of both chondrodystrophia foetalis and osteogenesis imperfecta. There were no demonstrable signs of cretinism. Nevertheless, the thyroid was almost completely transformed into fibrous tissue and was practically free from iodin. The weight of the pituitary body, however, was considerably in excess of the normal, in view of which Hektoen suggested that the effects of fibrous replacement of the thyroid had been compensated by increased secretory activity on the part of the hypophysis.

We have recently had occasion to study the anatomical peculiarities in five subjects of fetal chondrodystrophy — four times in infants and once in an adult — and have observed changes which, we believe, serve to establish the fact that a certain proportion of cases of chondrodystrophia foetalis in human beings is intimately associated with cretinism. Moreover, in every case we have been able to demonstrate histological alterations in the thyroid gland which suffice to account for the cretinistic phenomena.

In preparing our cases for publication we have omitted all reference to the several clinical histories, none of which contains information of value in the present connection, and the autopsy protocols have been abbreviated as much as is consistent with an intelligible presentation.

AUTOPSY PROTOCOLS OF AUTHORS' CASES

CASE 1.—Autopsy 3415. Post mortem examination by Drs. Wallace and Symmers.

The body was that of a female child, 23 months of age, 70 cm. in length. The stature was markedly abbreviated, squatty in type and the body presented a bloated appearance. The skin was tense, glistening and waxy, and the subdermal tissues were the seat of firm edema, most noticeable over the dorsum of the feet and in the region of the external genitalia. The head was markedly increased in size and dolichocephalic. It was 45 cm. in circumference at the level of the occipital protuberance. The eyes were widely separated and the eyelids, cheeks, lips and wings of the nostrils were greatly thickened. The abdomen was protuberant. The arms were shortened, measuring 26 cm. in length, the hypothenar eminences resting at the level of the crest of the ilium. The legs, which were large in caliber, measured 32 cm. in length. On section the subcutaneous tissues of the thoracic and abdominal walls presented the appearance of gelatinous edema. The loose tissues in the anterior mediastinum and around the kidneys were similarly involved. On stripping the muscles from the anterior thoracic wall a well-marked rosary came into view. This, on dissection, was found to consist of hyperplastic cartilages which overlapped the anterior extremi-

17. Cestan: Nouvelle Iconographie de la Salpêtrière, 1901, xiv, 277.
18. Christopher: American Med., June 7, 1902.
19. Hektoen: Am. Jour. Med. Sc., new series, 1903, cxxv, 751.

ties of the ribs and were specially conspicuous on the inner aspect of the chest. The foraman magnum was distinctly diminished in its antero-posterior diameter, the pelvis was contracted in all diameters and its bony elements were thickened. The long bones of both the upper and lower extremities were greatly shortened, the shafts were thickened and firm and the cartilages were enlarged and nodular. Thyroid: The thyroid weighed 4.4 gm. The left lobe was one-third again as large as the right. The gland was pinkish in color and firm in consistence. Pituitary: This organ was noticeably diminished in size. Lymphoid System: The lingual and faucial tonsils, the lymphoid follicles in the spleen and gastro-intestinal tract, including Peyer's patches, and the mesenteric lymph-nodes were markedly hyperplastic. Adrenals: Both adrenals were small and their cortices were narrowed. Aorta: This vessel was distinctly hypoplastic.

Anatomical Diagnosis: Chondrodystrophia foetalis; myxedema; chronic interstitial thyroiditis; status lymphaticus with concomitant hypoplasia of the adrenal cortex and of the aorta; bilateral hydrothorax, congestion and atelectasis of lungs; hypoplasia of pituitary.

Histological Examination of Thyroid: On histological examination of the thyroid it was found that the normal architecture was scarcely recognizable. The gland was traversed at numerous intervals by bands of dense, richly vascularized connective tissue intercommunicating in such a way as to divide the parenchyma into solid islands of varying shapes and sizes, the individual cells being closely packed. The parenchyma cells were pale and their cytoplasm was finely granular, while the nuclei varied in size and chromatic richness. In rare instances an alveolus was encountered which was comparable to the normal; that is to say, it was lined by a single layer of cuboidal epithelium, but colloid was practically absent. Lying in the connective tissue at scattered intervals were clumps of richly chromatic spindle-shaped cells. In places these cells did not confine themselves to the interstital framework, but encroached on and finally replaced the parenchymatous islands to such an extent that a low power field not infrequently was found to contain from ten to thirty alveoli in which the epithelium had been replaced by clumps of spindle cells standing out in strong contrast to the epithelial islands.

CASE 2.—Autopsy 3484. Section limited to abdominal incision. Post mortem examination by Dr. Symmers.

The body was that of a female child, 5 months of age, 53 cm. in length. The skin was pale and irregularly thrown into folds. The head was markedly enlarged and dolicocephalic. The upper eye-lids on both sides were puffy, giving a frog-like expression to the face. The nose was large, depressed at the root, and the wings of the nostrils, lips and cheeks were distinctly thickened. Both arms were shortened, the tips of the fingers reaching slightly below the level of the crest of the ilium. The abdomen was decidedly protuberant. On opening the abdomen a large amount of mucoid material of the appearance and consistence of Wharton's jelly was found in the loose connective tissue of the mesentery and of the gastro-hepatic omentum. Tongue: The tongue was enlarged and projected slightly beyond the lips. Thyroid: The thyroid measured 2.5 x 1.5 cm. and was coarsely lobulated. The organ was pinkish in color, the cut surface was flesh tinted and scattered over it were numerous minute grayish strands. No colloid was apparent to the naked eye. Lymphoid System: The lymphoid tissues were atrophic throughout.

Anatomical Diagnosis: Chondrodystrophia foetalis; cretinism; chronic interstitial thyroiditis; macroglossia; inanition.

Histological Examination of Thyroid: On examination of the thyroid gland it was practically impossible to identify the tissue from its microscopic appearances. The parenchyma was divided into large, variously shaped lobules by broad bands of coarsely fibrillated, poorly nucleated connective tissue from which delicate strands penetrated the cell groups at frequent intervals and subdivided them into small alveoli. The alveoli were crowded with large, flattened,

indistinctly outlined cells, the nuclei of which varied greatly in size and chromatic content, while the cytoplasm was scanty and finely granular. The majority of the nuclei were large, rounded and vesicular in appearance. Others were small and deeply staining. Of the nuclear changes, however, the most striking consisted in the great profusion of cells with huge, rounded, oval, or occasionally indented, nuclei which were rich in chromatin. In rarer instances these giant nuclei were vesicular in type. Mitotic figures were not found, colloid-containing vesicles were absent and the supporting tissues were poorly vascularized. In no instance did the parenchyma cells display any tendency to infiltrate the connective tissues at the periphery of the lobules.

CASE 3.—Autopsy 3503. Post mortem examination by Dr. Hagemeier.

The body was that of a male child, 15 months of age, 60.5 cm. in length. The head was markedly enlarged, measuring 46 cm. in circumference at the level of the parietal eminences, and the anterior fontanel was open and measured

Fig. 1.—Case 2.—High power drawing of thyroid showing the presence of alveoli surrounded by hyperplastic connective tissue and partially filled by epithelial cells of variable size and chromatic richness. Drawn with Edinger's apparatus.

5 x 3 cm. The ears were large and stood out prominently and the bridge of the nose was sunken. The arms were greatly shortened, measuring 30 cm. in length. The legs were pudgy and measured 20 cm. in length and the feet were broad. The abdomen was protuberant, measuring 38 cm. in circumference at the level of the umbilicus. There was a deep anterior curvature in the sacral region and a well formed thoracic rosary. Bony Skeleton: The anterior ends of the ribs were coarsely beaded, especially on the inner aspect. The sacrum projected into the pelvis to such an extent that the superior opening permitted the entrance of one finger only, while the lateral diameter corresponded to the breadth of two fingers. The foramen magnum was normal in size, but presented a tubercle springing from the posterior border into the spinal canal. Brain: The pia mater covering the upper surface of the cerebral hemispheres was thickened and gelatinous. Thyroid: The thyroid was described as "slightly enlarged, but otherwise

normal." Lymphoid System: This system presented the changes incident to well marked status lymphaticus associated with characteristic narrowing of the suprarenal cortices.

Anatomical Diagnosis: Chondrodystrophia foetalis; myxomatous transformation of the pia arachnoid; status lymphaticus.

Microscopic Examination of the Thyroid: Histological investigation of the thyroid revealed the presence of excessive over-growth of interstitial connective tissue, the trabeculae of which were disposed in such fashion as to divide the substance of the gland into irregular islands and to separate the individual

Fig. 2.—Case 4.—X-ray photograph of chondrodystrophic child, 2 months of age, 42 cm. in length, showing characteristic cartilaginous and bony changes. Note the enlarged cranial vault, the depressed nose, hyperplasia of the costochondral cartilages and those of the long bones, diminution in length and increase in thickness of the shafts of the bones of the extremities and the contracted pelvis.

alveoli one from the other. The connective tissue was moderately cellular and fairly well vascularized. For the greater part the alveoli were well formed and filled by colloid and varied in size within normal limits. They were lined by a single layer of well nucleated cuboidal epithelium. In occasional instances

groups of alveoli occurred the individuals of which were markedly compressed by connective tissue over-growth and were filled by small oval or spindle cells with densely chromatic nuclei.

CASE 4.—Autopsy 3055.[20] Post mortem examination by Dr. Norris.

The body was that of a female child, 2 months of age, 42 cm. in length. The head was large and measured 43 cm. in circumference. Both fontanels were open, the anterior measuring 5 x 6 cm. The eyes were widely separated and bulging and the lids were markedly thickened and corrugated. The tongue was large and protruded between the lips which, with the wings of the nostrils and the soft tissues of the ears, cheeks, forehead, neck and vulvae, were thickened. The arms were greatly shortened, measuring 15 cm. in length. The legs measured 20 cm. in length, the soft tissues of the dorsum of the feet were thickened and the toes were stubby. The skin of the forehead and of the upper thighs was thrown into coarse folds. The abdomen was markedly protuberant. On section the anterior chest wall exhibited a distinct rosary corresponding to hyper-

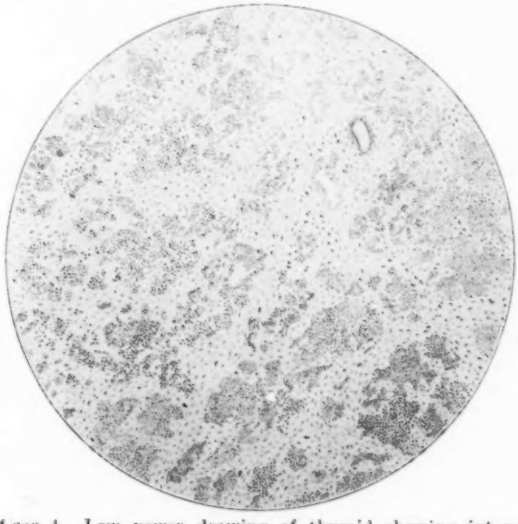

Fig. 3.—Case 4. Low power drawing of thyroid showing interstitial overgrowth and irregularly shaped alveoli filled by densely chromatic cells. Drawn with Edinger's apparatus.

plastic costal cartilages which were most conspicuous on the pleural side. The foramen magnum was very narrow in its anteroposterior diameter, the shafts of the long bones were short and thick and their cartilages were hyperplastic. Thyroid: The thyroid was very small, the lobes were symmetrical and weighed together 1 gm.

Anatomical Diagnosis: Chondrodystrophia foetalis; cretinism; atrophy of thyroid gland; atrophy of lymphoid system.

Histological Examination of Thyroid: On microscopic examination of the thyroid it was found that the normal architecture had been extensively altered and that the tissue could not be identified except for the presence of scattered groups of empty vesicles lined by very low cuboidal epithelium. The interstitial

20. For previous record of this case see La Fétra, Am. Jour. Dis. Child., 1913, v. 18.

tissues were markedly thickened and supported innumerable irregularly outlined cellular islands the individual constituents of which were densely packed and were provided with small, rounded or cylindrical, richly chromatic nuclei.

Skin: Microscopic examination of the skin showed marked atrophy of the epithelial papillae. The subdermal structures were greatly thickened and edematous and made up of coarse, swollen, hyalin fibers in which the nuclei were very small, few in number and poor in chromatin. In the deeper parts fat lobules occurred in abundance.

Muscle Tissue: Microscopic examination of the peripheral muscles revealed the existence of marked atrophy of the individual fibers with actual replacement at scattered intervals by clumps of cells with spindle shaped nuclei.

Case 5.—Autopsy 3445. Post mortem examination by Dr. Norris.

The body was that of a female, 31 years of age, 116 cm. in length. The head was large, brachycephalic, and measured 55 cm. in circumference. The nose was small, short and depressed at the bridge. The arms were abbreviated, measuring 38 cm. in length and the hands, which were small, did not reach beyond the crest of the ilium. The legs measured 43.5 cm. in length. The abdomen was protuberant and the subcutaneous fat was very abundant. Further examination of the body revealed nothing worthy of mention other than characteristic hyperplastic changes in the cartilages of the long bones, thickening and sclerosis of the shafts and diminution in the anteroposterior diameter of the foramen magnum. The thyroid weighed 6.5 gm. and was deep red in color.

Anatomical Diagnosis: Chondrodystrophia foetalis; chronic parenchymatous nephritis; fatty degeneration of the heart and liver; acute pharyngitis; fibromyoma of uterus; sclerosis of ovaries; congestion of thyroid.

Histological Examination of Thyroid: On microscopic examination of the thyroid it was found that the parenchyma was irregularly divided into large islands by broad bands of coarsely fibrillar, poorly nucleated connective tissue. Delicate subsidiary trabeculae penetrated the islands and surrounded the individual alveoli. In the connective tissue framework deeply injected vessels were numerous. In the larger trabeculae the vessels were of considerable size, while the more delicate strands around the alveoli supported a vascular net-work the ramifications of which included practically every alveolus in a capillary loop, so that the contents of the vessels were separated from the epithelium of the alveoli by a single layer of endothelium. The alveoli varied in size within moderate limits. The smallest were free from colloid and scarcely larger than those encountered in the fetal gland, while the largest approximated in size the alveoli of the adult thyroid and were partially or completely filled by faintly staining colloid. Although the smaller alveoli predominated, the larger, colloid-containing vesicles were present in considerable numbers. The epithelial cells in the alveoli were fairly large, oval or rounded, and were provided with moderately chromatic nuclei and finely granular, faintly acidophilic cytoplasm. The larger alveoli were lined by a single layer of cells, while the smaller were partially or completely filled by detached epithelium. In addition to these changes there occurred in the connective tissue at scattered intervals small foci of cells with richly chromatic, spindle-shaped nuclei and scarcely perceptible cytoplasm. In no instance was it possible to detect any sign of invasion of the alveoli by these cells.

CONCLUSIONS

1. There is a form of chondrodystrophia foetalis in which, in addition to changes in the osseous system, we feel justified in emphasizing the occurrence of modifications in the soft tissues which are attributable to pathological defects in the thyroid gland. The modifications in question consist of thickening of the lips, cheeks and eyelids, the wings of the

nostrils and the lobes of the ears, macroglossia, hypertrophic vulvae and myxomatous transformation of the subcutaneous and certain deep connective tissues, all of which, when combined with the large head and frog-like expression, the protuberant abdomen, prominent skin folds and pudgy extremities, fulfill the essential requirements for the diagnosis of cretinism. In the latter connection it is to be observed that the chondrodystrophic individual very often bears the so-called "cretinoid physiognomy" without presenting any evidence of cretinism. In these circumstances the large head, depressed nose and dwarfed body, while constituting a superficial resemblance to cretinism are, in reality, the outward expression of a disease which is referable to the bony system and which arises independently of demonstrable changes in the thyroid gland. To

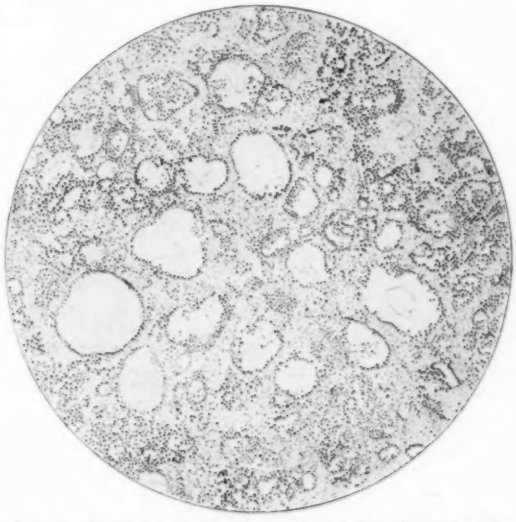

Fig. 4.—Case 5. Low power drawing of thyroid showing extensive connective tissue hyperplasia, alveoli partially or completely filled by detached epithelium, and colloid-containing vesicles. Female, aged 31. Drawn with Edinger's apparatus.

what extent, if any, functional disturbances in the developing thyroid are responsible for the skeletal deformities is an open question. When chondrodystrophia foetalis and cretinism coexist the picture becomes still further involved, and it may not always be apparent to what extent the physiognomy is determined by cretinism and how much of it is due to the chondrodystrophic process acting through premature synostosis of the os tribasilare.

2. In the cretinistic variety of chondrodystrophia foetalis which we have just described, the pathological changes in the thyroid gland are

characterized by an extensive chronic productive inflammatory process eventuating in replacement of the colloid vesicles by means of over-growth of alveolar epithelium, or by invasion and substitution of the alveoli by connective tissue elements derived from the interstitium, aided, in both instances, by compression exerted from the outside by the contracting fibrous trabeculae.

3. In Case 5 we are confronted by an instance of chondrodystrophia foetalis in a female 31 years of age, whose thyroid gland showed the histological changes incident to chronic interstitial thyroiditis. While most of the vesicles were free from colloid, considerable numbers were filled or even slightly distended. Unfortunately we are unable to give any information concerning the colloid content of the pituitary body. At the post mortem examination it was noted that the physiognomy was "cretinoid" in type, but signs of myxedema were not detected. In the absence of a history of cretinism in childhood we must yield to the natural conclusion that, during life, the patient's thyroid, although extensively diseased, was sufficiently active to protect her against myxedema.

4. In Case 3 the subject was a child, 15 months of age, in whose bony skeleton the signs of fetal chondrodystrophy were indisputable, but the changes indicative of cretinism were limited to myxomatous transformation of the pia-arachnoid and to enlargement of the ears, pudgy legs and spade-like hands. The thyroid was described as "slightly enlarged," and histological examination showed evidences of an extensive chronic interstitial thyroiditis in which, however, colloid-containing vesicles occurred in large numbers. Here, again, we must conclude that the child was, to an extent, protected against myxedema as the result of partial preservation of the functional activity of the thyroid gland.

In conclusion, we wish to acknowledge our gratitude to Dr. Charles Norris for his kindly interest and cooperation, and to thank Dr. K. D. Bryson for aid in the preparation of the illustrations. Acknowledgement is also due Drs. La Fétra and Lindeman.

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STUDY XVII: EXPERIMENTAL CHRONIC NEPHRITIS PRODUCED BY THE COMBINATION OF CHEMICAL (URANIUM NITRATE) AND BACTERIA (*B. COLI COMMUNIS*)*

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This study has been made at the suggestion of Dr. Henry A. Christian in an attempt to produce a chronic nephritis in animals in a manner comparable to that used by Opie[1] in the production of an experimental cirrhosis of the liver. Opie found that repeated injections of chloroform, which causes a necrosis of the liver, and *Bacillus coli communis*, whose relation to the liver is quite intimate, produced a well-marked sclerosis of the liver. In the present experiments repeated injections of uranium nitrate and *B. coli communis* were made in rabbits to see if in similar way a sclerosis of the kidney would be produced. Uranium nitrate is known to produce an acute nephritis similar to that occurring in human beings in which there is very extensive necrosis of the tubular epithelium, and has been shown by Dickson,[2] Smith[3] and others, to produce a chronic nephritis when injected repeatedly over a long period of time. Although the relationship between *B. coli communis* and the kidney is not as intimate as that with the liver, infection of the urinary tract with colon bacilli is fairly common and it has been suggested that these bacteria may play a part in the chronic nephritis occurring in man.

Rabbits were the animals used. Although full grown rabbits were selected in most of the experiments, it was found very difficult to keep them alive long enough to produce any chronic lesion. In fact, about one-half of all the rabbits started, died within a few days, either from a septicemia from *B. coli communis* or from the uranium nitrate, or from both. Only eighteen out of forty rabbits used, survived long enough to produce any chronic lesions. These eighteen were carried through a

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* A series of studies on experimental cardiovascular disease has been published previously: see Study XVI, THE ARCHIVES OF INT. MED., 1913, xi, 517, for reference to these.

* From the Laboratory of the Department of the Theory and Practice of Physic, Medical School, Harvard University.

* This work was done under a grant from the Proctor Fund for the Study of Chronic Diseases.

* Submitted for publication March 25, 1913.

1. Opie: Jour. Exper. Med., 1909, xii, 237.
2. Dickson: THE ARCHIVES INT. MED., 1909, iii, 375.
3. Smith: THE ARCHIVES INT. MED., 1911, viii, 481.

period of life varying from a few weeks to five months. Some died, most of them were killed.

The exact method employed varied with the individual animal and can be found in the protocols. One was given 5 mg. of uranium nitrate subcutaneously every three weeks, and at the same time 0.25 c.c. of a twenty-four hour bouillon culture of *B. coli communis* intravenously every three days. Four were given 2 mg. uranium nitrate at intervals of three days for three doses, followed by three doses of 0.25 c.c. of a twenty-four hour bouillon culture of *B. coli communis* at three-day intervals, etc. The rest were given alternate doses of uranium nitrate 2 mg., and *B. coli communis* 0.25 c.c. at three-day intervals. In one

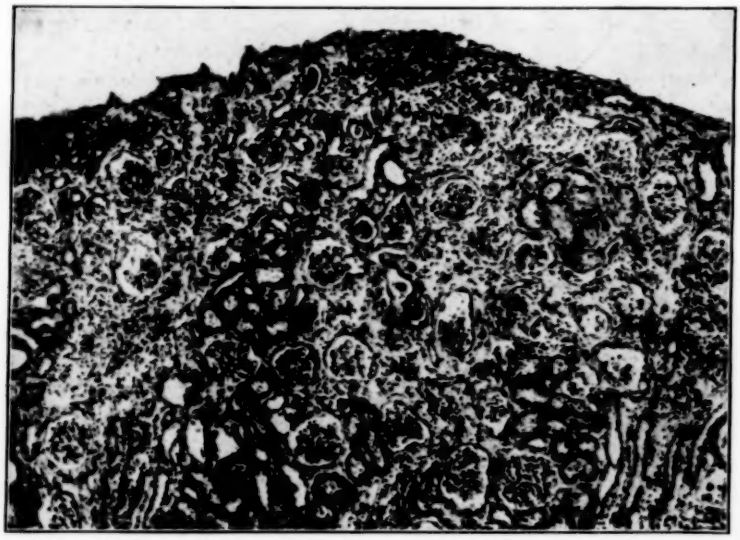

Fig. 1.—Rabbit 370. Magnification 65 diameters. Kidney showing area of connective tissue increase radiating into the cortex from the surface.

case, Rabbit 370, the dose of *B. coli communis* was increased during the last two months to 0.35 c.c.

The results of this treatment were that twelve out of the eighteen rabbits showed a moderately marked increased of the connective tissue, four a moderate increase, two a slight increase in the connective tissue. In thirteen the connective tissue was present in the outer cortex, usually starting from the surface in form of a scar running down one or more of the rays. (Fig. 1, Rabbit 370; Fig. 2, Rabbit 445; Fig. 3, Rabbit 461; Fig. 4, Rabbit 472.) In two of these cases the connective tissue was very marked in the outer cortex. In seventeen of the cases there was increased

fibrous tissue at the junction between the cortex and the medulla. (Fig. 5, Rabbit 471; Fig. 6, Rabbit 473; Fig. 7, Rabbit 460.) This seemed to be the favorite place for the greatest increase, as thirteen of the eighteen cases showed a marked increase in this region. Fourteen cases showed an increase of fibrous tissue in the medulla; in none was the increase very marked.

The connective tissue was quite fibrous in most cases, though in some it was faily cellular. In most of the kidneys ail three localities were involved, the connective tissue spreading from the surface down the rays in form of scars containing distorted glomeruli. These scars were con-

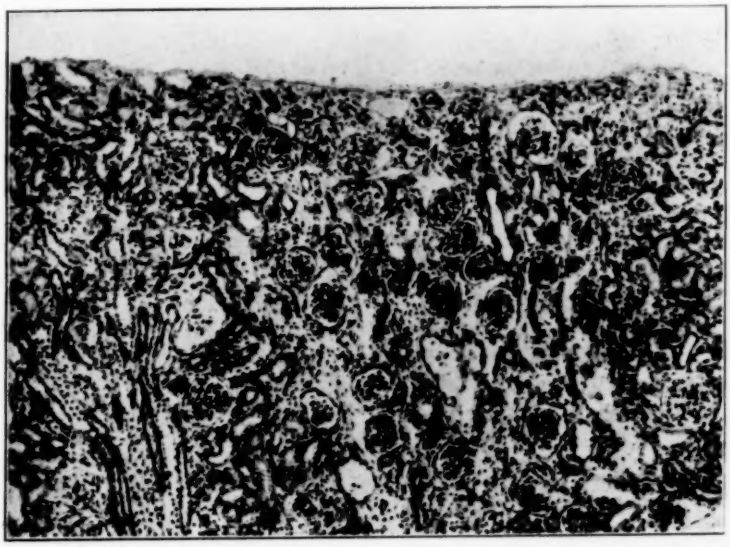

Fig. 2.—Rabbit 445. Magnification 65 diameters. Kidney showing area of connective tissue increase radiating into the cortex from the surface.

nected at the apex of the labyrinths by a thick band of connective tissue running parallel to the surface. The connective tissue in the medulla was never very marked.

Lymphocytic infiltration was present in ten of the eighteen cases, but was well marked in only three of the cases. No special part of the kidney was singled out, clumps of small lymphocytes being present here and there in all parts.

Most of the cases showed involvement of the glomeruli, this taking one or more of several forms. Ten showed distinct drawing together of the glomeruli by contraction of the connective tissue in the scars. (Figs.

1, 2 and 3.) Six showed a more or less marked dilatation of the capsule, usually accompanied by a shrinking of the tuft as from pressure of fluid in the capsular space. (Fig. 3.) Five showed shrinking of the glomeruli and twelve a distinct thickening of the capsular walls of many glomeruli. Ten showed also a thickening of the walls of the vessels in the tuft. Four showed a proliferation of the endothelial cells in the glomeruli and four an increased number of red blood-corpuscles in the glomeruli. Only seven out of the eighteen showed any dilatation of tubules such as Smith[3] speaks about. This is only well marked in two cases. (Fig. 8, Rabbit 442.)

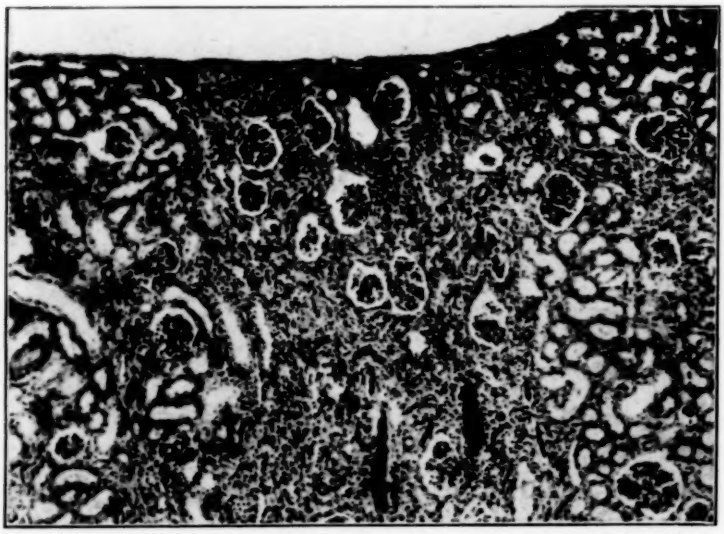

Fig. 3.—Rabbit 461. Magnification 65 diameters. Kidney showing area of connective tissue increase radiating into the cortex from the surface.

In comparing the results of this series of experiments with those obtained by Smith[3] a general impression was obtained that chronic changes were more regularly encountered in the kidneys of rabbits which had received both uranium nitrate and *Bacillus coli communis* than in those rabbits which had received only uranium nitrate. It was found difficult, however, to make an actual comparison between the lesions in two sets, inasmuch as the rabbits of Smith's series of experiments varied considerably in the method of treatment from the rabbits in this series, both with regard to the dosage and the time elapsing between the last dose and the time of death.

There were, however, ten rabbits which were carried through an approximately equal period of active treatment. In five uranium nitrate alone was given; in the other five alternate doses of uranium nitrate and *Bacillus coli communis* were given, the total number of doses of the two being approximately the same as the total number of doses of uranium nitrate in the first five. In these rabbits the amount of connective tissue change showed no great preponderance in either set. On the whole, the connective tissue was rather more dense in the kidneys of the rabbits which had received both uranium nitrate and *Bacillus coli communis*. In addition to these ten rabbits, there were six rabbits, all of which had

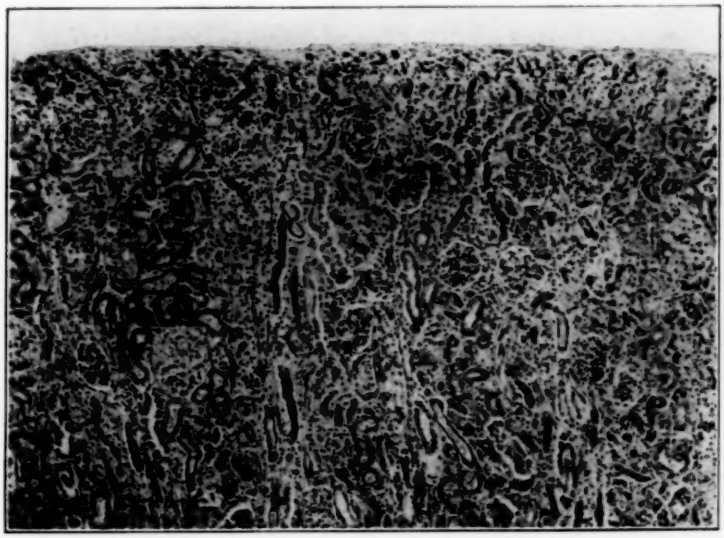

Fig. 4.—Rabbit 472. Magnification 65 diameters. Kidney showing area of connective tissue increase radiating into the cortex from the surface.

received approximately the same number of equal size doses of uranium nitrate over a period of equivalent time. Three of these in addition had received doses of *Bacillus coli communis*. In this set the rabbits that had received both uranium nitrate and *Bacillus coli communis* showed distinctly a more marked connective tissue change in their kidneys.

In any study of experimental chronic nephritis in the rabbit it must be kept in mind that the kidney of the rabbit very frequently shows a slight amount of connective tissue which has arisen under natural conditions in the animal, and is not the result of any form of experimental treatment. Occasionally quite marked chronic lesions are encountered

in these untreated animals. Making due allowance for these spontaneous lesions and utilizing as controls the tissues of the kidneys of those rabbits in the series which died of acute lesions very shortly after the beginning of the treatment, it would appear that the combination of uranium nitrate and *Bacillus coli communis* very definitely and quite regularly produces in the rabbit's kidney a chronic lesion with connective tissue increase, and other changes in many respects comparable to the lesions of chronic interstitial nephritis in man in so far as renal elements are concerned. The arteriosclerotic lesions that are so common in the human kidneys of chronic interstitial nephritis are not found in these rabbit kidneys.

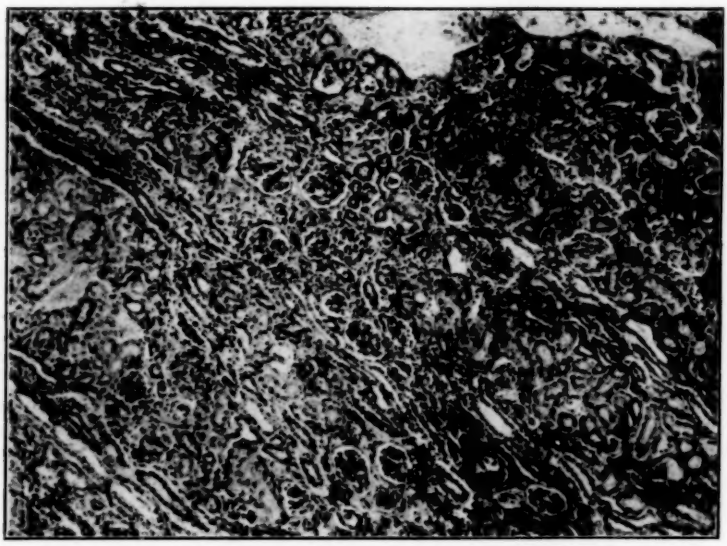

Fig. 5.—Rabbit 471. Magnification 65 diameters. Kidney showing connective tissue increase at the junction between cortex and medulla.

In the series of kidneys described in this paper it would seem fair to exclude those kidneys described as showing a moderate or slight increase in connective tissue change, inasmuch as these changes, though rather more frequent in this series, were not in excess of the lesions very commonly found in untreated rabbits. This, then, would leave twelve rabbits in which a considerable increase in connective tissue was produced in the kidney after injections of uranium nitrate and *Bacillus coli communis* covering a very considerable period of time as shown in the protocols. In other words, of 40 rabbits used in these experiments, 22 died so soon after the first injections as to exclude the possibility of any

chronic lesion; 18 lived long enough under treatment to show possible chronic lesions. Of these 18, 12 or two-thirds, showed a considerable degree of chronic change, sufficient to justify the term chronic experimental nephritis. The remaining 6, though showing some chronic change, are excluded from the series inasmuch as the change shown was not more than is frequently seen in rabbits not treated.

The protocols of the rabbits showing more definite changes are appended.

PROTOCOLS

RABBIT 366.—Weight, 1,650 grams. Uranium nitrate subcutaneously 3 mg., Dec. 4, 1911; 5 mg., December 9; 3 mg., December 11; 5 mg., December 13. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, December

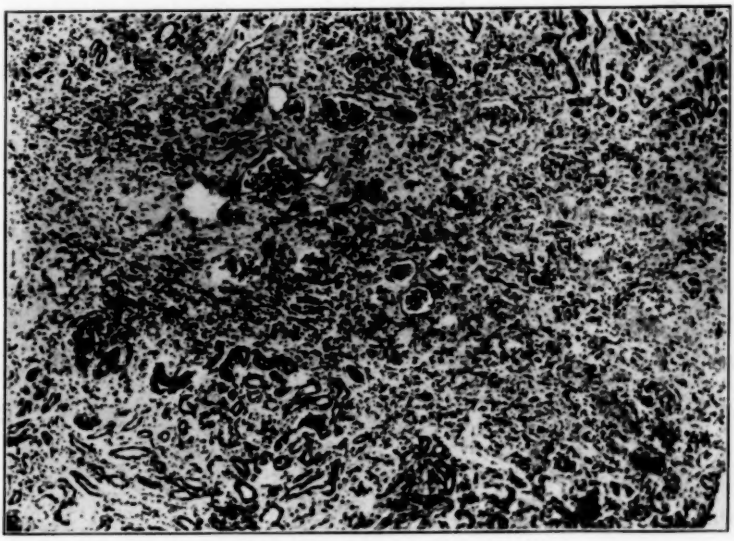

Fig. 6.—Rabbit 473. Magnification 65 diameters. Kidney showing connective tissue increase at the junction between cortex and medulla.

15, 18, 21. Uranium nitrate subcutaneously 2 mg., December 24, 27, 30. *B. coli communis* intravenously 0.25 c.c., Jan. 2, 5, 8, 1912. Uranium nitrate subcutaneously 2 mg., January 11, 14, 17. *B. coli communis* intravenously 0.25 c.c., January 20, 23, 26. Uranium nitrate subcutaneously 2 mg., January 29, February 1, 4. *B. coli communis* intravenously 0.25 c.c., February 7, 10, 13. Uranium nitrate 2 mg. subcutaneously, February 16, 19, 22. *B. coli communis* intravenously 0.35 c.c., February 25, 28, March 2. Uranium nitrate subcutaneously 2 mg., March 5, 7, 10.

Rabbit killed March 12, 1912. Duration of experiment seventy days. Kidney shows moderate connective tissue increase most marked in mid-zonal region.

RABBIT 370.—Caged Dec. 21, 1911. Urine negative. Uranium nitrate subcutaneously 2 mg., December 22, 24, 27, 30. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, Jan. 2, 5, 8, 1912. Uranium nitrate sub-

Fig. 7.—Rabbit 460. Magnification 65 diameters. Kidney showing connective tissue increase at the junction between cortex and medulla.

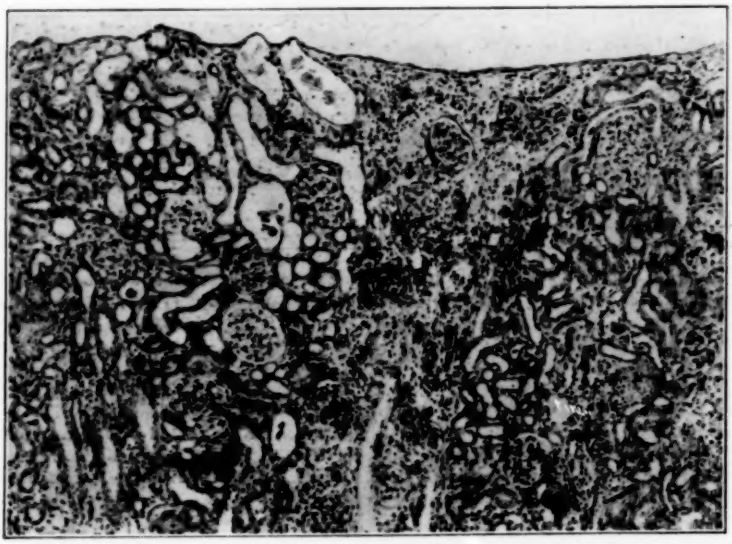

Fig. 8.—Rabbit 442. Magnification 65 diameters. Kidney showing connective tissue radiating into the cortex from the surface with dilatation of adjacent renal tubules.

cutaneously 2 mg., Jan. 11, 14, 17. *B. coli communis* intravenously 0.25 c.c., January 20, 23, 26. Uranium nitrate subcutaneously 2 mg., Jan. 29, February 1, 4. *B. coli communis* intravenously 0.25 c.c., February 7, 10, 13. Uranium nitrate subcutaneously 2 mg., February 16, 19, 22. *B. coli communis* intravenously 0.35 c.c., February 25, 28, March 2. Uranium nitrate subcutaneously 2 mg., March 5, 7, 10. *B. coli communis* intravenously 0.35 c.c., March 15, 18, 21. Uranium nitrate subcutaneously 2 mg., March 24, 27, 30. *B. coli communis* intravenously 0.35 c.c., April 2, 5, 8. Uranium nitrate subcutaneously 2 mg., April 11, 14, 17. *B. coli communis* intravenously 0.35 c.c., April 21.

Killed April 24, 1912. Duration of experiment 126 days. Kidney shows bands radiating in from the surface in which there is a moderate connective tissue increase, rather cellular in character.

RABBIT 382.—Weight, 1,400 grams. Caged Dec. 28, 1911. Urine negative. Uranium nitrate subcutaneously 3.33 mg. per K., December 30, 31. *B. coli communis* intravenously 0.25 c.c. of a 24-hour bouillon culture, January 27. Uranium nitrate subcutaneously 5 mg., February 17. *B. coli communis* intravenously 0.25 c.c., March 9. Uranium nitrate subcutaneously 5 mg. and *B. coli communis* intravenously 0.25 c.c., March 30. *B. coli communis* intravenously 0.25 c.c., April 2, 5, 8, 11, 14, 18. Uranium nitrate subcutaneously 5 mg. and *B. coli communis* intravenously 0.25 c.c., April 21. *B. coli communis* intravenously 0.25 c.c., April 24, 27, 30, May 4, 7, 10, 13, 15, 17, 20, 23, 26, 29, June 1. Uranium nitrate subcutaneously 5 mg. and *B. coli communis* intravenously 0.25 c.c., June 4. *B. coli communis* intravenously 0.25 c.c., June 7.

Killed June 10, 1912. Duration of experiment 165 days. Kidney shows condition quite similar to Rabbit 366.

RABBIT 440.—Caged March 3, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 24. *B. coli communis* intravenously 0.25 c.c., of 24-hour bouillon culture, March 27. Uranium nitrate subcutaneously 2 mg., March 30. *B. coli communis* intravenously 0.25 c.c., April 2. Uranium nitrate subcutaneously 2 mg., April 5. *B. coli communis* intravenously 0.25 c.c., April 14. Uranium nitrate subcutaneously 2 mg., April 18. *B. coli communis* intravenously 0.25 c.c, April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 30. *B. coli communis* intravenously 0.25 c.c., May 24. Uranium nitrate subcutaneously 2 mg., May 7. *B. coli communis* intravenously 0.25 c.c., May 10. Uranium nitrate subcutaneously 2 mg., May 13. *B. coli communis* intravenously 0.25 c.c., May 17. Uranium nitrate subcutaneously 2 mg., May 20. *B. coli communis* intravenously 0.25 c.c., May 23. Uranium nitrate subcutaneously 2 mg., May 26. *B. coli communis* intravenously 0.25 c.c., May 29. Uranium nitrate subcutaneously 2 mg., June 1. *B. coli communis* intravenously 0.25 c.c., June 4. Uranium nitrate subcutaneously 2 mg., June 7. *B. coli communis* intravenously 0.25 c.c., June 10. Uranium nitrate subcutaneously 2 mg., June 13. *B. coli communis* intravenously 0.25 c.c, June 16, etc. *B. coli communis* intravenously 0.25 c.c., June 26.

Killed July 8, 1912. Duration of experiment 106 days. Kidney shows slight focal increase in connective tissue, cellular in type.

RABBIT 442.—Weight, 1,560 grams. Caged March 9, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 10, 13, 16. *B. coli communis* intravenously 0.25 c.c. of a 24-hour bouillon culture, March 18, 21, 24. Uranium nitrate subcutaneously 2 mg., March 27, 30, April 2. *B. coli communis* intravenously 0.25 c.c., April 5, 8, 11. Uranium nitrate subcutaneously 2 mg., April 14, 17, 21. *B. coli communis* intravenously 0.25 c.c., April 24, 27, 30. Uranium nitrate subcutaneously 2 mg., May 4, 7, 10. *B. coli communis* intravenously 0.25

c.c., May 13, 17, 20. Uranium nitrate subcutaneously 2 mg., May 23, 26, 29. *B. coli communis* intravenously June 1, 4, 7.

Killed June 10, 1912. Duration of experiment 93 days. Kidney shows scattered areas of connective tissue increase with distinct dilatation of some of tubules.

RABBIT 445.—Weight, 1,530 grams. Caged March 10, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 11, 14, 16. *B. coli communis* intravenously 0.25 c.c. of a 24-hour bouillon culture, March 18, 21, 24. Uranium nitrate subcutaneously 2 mg., March 27, 30, April 2. *B. coli communis* intravenously 0.25 c.c., April 5, 8, 11. Uranium nitrate subcutaneously 2 mg., April 14, 17, 21. *B. coli communis* intravenously 0.25 c.c., April 24, 27, 30. Uranium nitrate subcutaneously 2 mg., May 4, 7, 10. *B. coli communis* intravenously 0.25 c.c., May 13, 17, 20. Uranium nitrate subcutaneously 2 mg., May 23, 26, 29. *B. coli communis* intravenously 0.25 c.c., June 1, 4, 7.

Killed June 10, 1912. Duration of experiment 92 days. Kidney shows quite large areas in which there is a very considerable connective tissue increase.

RABBIT 460.—Weight, 2,380 grams. Caged March 27, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 27. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, March 30. Uranium nitrate subcutaneously 2 mg., April 2. *B. coli communis* intravenously 0.25 c.c., April 5. Uranium nitrate subcutaneously 2 mg., April 8. *B. coli communis* intravenously 0.25 c.c., April 11. Uranium nitrate subcutaneously 2 mg., April 14. *B. coli communis* intravenously 0.25 c.c., April 17. Uranium nitrate subcutaneously 2 mg., April 21. *B. coli communis* intravenously 0.25 c.c., April 24. Uranium nitrate subcutaneously 2 mg., April 27. *B. coli communis* intravenously 0.25 c.c., April 30. Uranium nitrate subcutaneously 2 mg., May 4. *B. coli communis* intravenously 0.25 c.c., May 7. Uranium nitrate subcutaneously 2 mg., May 10. *B. coli communis* intravenously 0.25 c.c., May 13. Uranium nitrate subcutaneously 2 mg., May 17. *B. coli communis* intravenously 0.25 c.c., May 20. Uranium nitrate subcutaneously 2 mg., May 23. *B. coli communis* intravenously 0.25 c.c., May 26. Uranium nitrate subcutaneously 2 mg., May 29. *B. coli communis* intravenously 0.25 c.c., June 1. Uranium nitrate subcutaneously 2 mg., June 16. *B. coli communis* intravenously 0.25 c.c., June 20. Uranium nitrate subcutaneously 2 mg., June 23. *B. coli communis* intravenously 0.25 c.c., June 26. Uranium nitrate subcutaneously 2 mg., June 29.

Killed July 8, 1912. Duration of experiment 103 days. Kidney shows picture quite similar to Rabbit 445.

RABBIT 461.—Weight, 2,100 grams. Caged March 27, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 27. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, March 30. Uranium nitrate subcutaneously 2 mg., April 2. *B. coli communis* intravenously 0.25 c.c., April 5. Uranium nitrate subcutaneously 2 mg., April 8. *B. coli communis* intravenously 0.25 c.c., April 11. Uranium nitrate subcutaneously 2 mg., April 14. *B. coli communis* intravenously 0.25 c.c., April 18. Uranium nitrate intravenously 2 mg., April 21. *B. coli communis* intravenously 0.25 c.c., April 24. Uranium nitrate subcutaneously 2 mg., April 27. *B. coli communis* intravenously 0.25 c.c., April 30. Uranium nitrate subcutaneously 2 mg., May 4. *B. coli communis* intravenously 0.25 c.c., May 7. Uranium nitrate subcutaneously 2 mg., May 10. *B. coli communis* intravenously 0.25 c.c., May 13. Uranium nitrate subcutaneously 2 mg., May 17. *B. coli communis* intravenously 0.25 c.c., May 20. Uranium nitrate subcutaneously 2 mg., May 23. *B. coli communis* intravenously 0.25 c.c., May 26. Uranium nitrate subcutaneously 2 mg., May 29. *B. coli communis* intravenously 0.25 c.c., June 1. Uranium nitrate subcutaneously 2 mg., June 4. *B. coli communis* intravenously 0.25 c.c., June 7. Uranium nitrate subcutaneously 2 mg., June 10. *B. coli communis* intravenously 0.25 c.c., June 13. Uranium nitrate subcutaneously 2 mg., June 16. *B. coli communis* intravenously 0.25 c.c., June 20. Uranium nitrate subcutaneously 2 mg., June 23. *B. coli*

communis intravenously 0.25 c.c., June 26. Uranium nitrate subcutaneously 2 mg., June 29.

Killed July 8, 1912. Duration of experiment ninety-eight days. Kidney shows areas radiating in from surface in which there is considerable connective tissue increase, cellular in character with almost complete disappearance of tubules.

RABBIT 468.—Weight, 1,890 grams. Caged April 10, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 11. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, April 14. Uranium nitrate subcutaneously 2 mg., April 18. *B. coli communis* intravenously 0.25 c.c., April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 30. *B. coli communis* intravenously 0.25 c.c., May 4. Uranium nitrate subcutaneously 2 mg., May 7. *B. coli communis* intravenously 0.25 c.c., May 10. Uranium nitrate subcutaneously 2 mg., May 13. *B. coli communis* intravenously 0.25 c.c., May 17. Uranium nitrate subcutaneously 2 mg., May 20. *B. coli communis* intravenously 0.25 c.c., May 23. Uranium nitrate subcutaneously 2 mg., May 26. *B. coli communis* intravenously 0.25 c.c., May 29. Uranium nitrate subcutaneously 2 mg., June 1. *B. coli communis* intravenously 0.25 c.c., June 4. Uranium nitrate subcutaneously 2 mg., June 7. *B. coli communis* intravenously 0.25 c.c., June 10. Uranium nitrate subcutaneously 2 mg., June 13. *B. coli communis* intravenously 0.25 c.c., June 16. Uranium nitrate subcutaneously 2 mg., June 20. *B. coli communis* intravenously 0.25 c.c., June 23. Uranium nitrate subcutaneously 2 mg., June 26. *B. coli communis* intravenously 0.25 c.c., June 29.

Killed July 8, 1912. Duration of experiment 89 days. Kidney shows moderate degree of connective tissue increase, more marked in mid-zonal region and fairly cellular.

RABBIT 470.—Weight, 1,820 grams. Caged April 10, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 11. *B. coli communis* intravenously 0.25 c.c., of 24-hour bouillon culture, April 14. Uranium nitrate subcutaneously 2 mg., April 17. *B. coli communis* intravenously 0. 25 c.c., April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 30. *B. coli communis* intravenously 0.25 c.c., May 4. Uranium nitrate subcutaneously 2 mg., May 7, etc., to June 10.

Found dead June 10, 1912. Duration of experiment fifty-nine days. Kidney shows a moderate degree of connective tissue increase quite diffuse in its distribution.

RABBIT 471.—Weight, 1,912 grams. Caged April 10, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 11. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, April 14. Uranium nitrate subcutaneously 2 mg., April 17. *B. coli communis* intravenously 0.25 c.c., April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 30. *B. coli communis* intravenously 0.25 c.c., May 4. Uranium nitrate subcutaneously 2 mg., May 7. *B. coli communis* intravenously 0.25 c.c., May 10. Uranium nitrate subcutaneously 2 mg., May 13. *B. coli communis* intravenously 0.25 c.c., May 17. Uranium nitrate subcutaneously 2 mg., May 20. *B. coli communis* intravenously 0.25 c.c., May 23. Uranium nitrate subcutaneously 2 mg., May 26. *B. coli communis* intravenously 0.25 c.c., May 29. Uranium nitrate subcutaneously 2 mg., June 1. *B. coli communis* intravenously 0.25 c.c., June 4. Uranium nitrate subcutaneously 2 mg., June 7. *B. coli communis* intravenously 0.25 c.c., June 10. Uranium nitrate subcutaneously 2 mg., June 13. *B. coli communis* intravenously 0.25 c.c., June 16.

Found dead June 20, 1912. Duration of experiment sixty-nine days. Kidney shows condition quite similar to Rabbit 470, but more marked in degree.

RABBIT 472.—Weight, 1,450 grams. Caged April 7, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 11. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, April 14. Uranium nitrate subcutaneously 2 mg., April 18. *B. coli communis* intravenously 0.25 c.c., April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 20. *B. coli communis* intravenously 0.25 c.c., May 4. Uranium nitrate subcutaneously 2 mg., May 7. *B. coli communis* intravenously 0.25 c.c., May 10. Uranium nitrate subcutaneously 2 mg., May 13. *B. coli communis* intravenously 0.25 c.c., May 17. Uranium nitrate subcutaneously 2 mg., May 20. *B. coli communis* intravenously 0.25 c.c., May 23. Uranium nitrate subcutaneously 2 mg., May 26. *B. coli communis* intravenously 0.25 c.c., May 29. Uranium nitrate subcutaneously 2 mg., June 1. *B. coli communis* intravenously 0.25 c.c., June 4.

Found dead June 5, 1912. Duration of experiment fifty-four days. Kidney shows condition quite similar to Rabbit 471.

RABBIT 473.—Caged April 20, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 21. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, April 24. Uranium nitrate subcutaneously 2 mg., April 27. *B. coli communis* intravenously 0.25 c.c., April 30. Uranium nitrate subcutaneously 2 mg., May 4. *B. coli communis* intravenously 0.25 c.c., May 7. Uranium nitrate subcutaneously 2 mg., May 10. *B. coli communis* intravenously 0.25 c.c., May 13. Uranium nitrate subcutaneously 2 mg., May 17. *B. coli communis* intravenously 0.25 c.c., May 20. Uranium nitrate subcutaneously 2 mg., May 23. *B. coli communis* intravenously 0.25 c.c., May 26. Uranium nitrate subcutaneously 2 mg., May 29. *B. coli communis* intravenously 0.25 c.c, June 1. Uranium nitrate subcutaneously 2 mg., June 4. *B. coli communis* intravenously 0.25 c.c., June 7. Uranium nitrate subcutaneously 2 mg., June 10. *B. coli communis* intravenously 0.25 c.c., June 13. Uranium nitrate subcutaneously 2 mg., June 16. *B. coli communis* intravenously 0.25 c.c., June 20.

Found dead June 21, 1912. Duration of experiment sixty-three days. Kidney shows quite marked connective tissue increase, diffuse in character and in addition areas of marked connective tissue increase radiating in from surface in which tubules have practically disappeared.

CONCLUSIONS

In rabbits that survive the immediate toxic effects of the agents employed, chronic renal lesions can be produced with considerable regularity by injections of a chemical (uranium nitrate) and a bacteria (*B. coli communis*). The lesions so produced are closely similar to those involving the renal tissues in chronic interstitial nephritis in man.

Carney Hospital.

STUDY XVIII: ACUTE RENAL LESIONS PRODUCED BY URANIUM NITRATE IN THE DOG IN COMPARISON WITH THE RABBIT *

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In 1908, Christian[1] described the occurrence of hyaline droplets in the walls of the capillaries of the glomerular tuft in rabbits that had recently received doses of uranium nitrate. In 1911, a further study of this lesion was published.[2] At Dr. Christian's suggestion, I undertook to see whether a similar lesion could be produced in dogs by injection of uranium nitrate.

Nine dogs of varying breeds and weights were tested by urinary examination to exclude a possible spontaneous nephritis. They then were given subcutaneous injections of uranium nitrate. The number of doses and their time interval differed considerably, varying from a single dose of 3.3 mg. per kilo of body weight to three doses of 12 mg. per kilo of body weight. Except in two instances, the doses were given on successive days, and the animal killed twenty-four hours after the last dose by primary chloroform anesthesia and deflation of the lungs by incision of the diaphragm.

A study of the renal tissues shows that the hyaline droplets are much more difficult of production in dogs than in rabbits, and when they do occur as a rule, they are not confined so definitely to the wall of the capillary in the glomerular tuft. In the rabbit two or three doses of uranium nitrate 3.3 mg. per kilo of body weight, or larger, quite regularly produced extensive renal lesions, among them hyaline droplets in many of the glomeruli. In the dog, even with large doses of uranium nitrate, this glomerular lesion was infrequent, and with smaller doses such as produced in the rabbits the hyaline droplets with great regularity and in typical form, in the dog they did not occur. The epithelium of the tubules in the dog's kidney shows the same lesion as that found in the rabbit, degeneration or necrosis, with disintegration of the renal epith-

* A series of studies on experimental cardiovascular disease has been published previously; see Study XVI, THE ARCHIVES OF INT. MED., 1913, xi, 517, for reference to these.

* From the Laboratory of the Department of the Theory and Practice of Physic, Medical School, Harvard University.

* This work was done under a grant from the Proctor Fund for the Study of Chronic Diseases.

1. Christian, H. A.: A Glomerular Lesion of Experimental Nephritis. Boston Med. and Surg. Jour., 1908, clix.

2. Christian, H. A.: THE ARCHIVES OF INT. MED., 1911, viii, 469.

elium; but here again larger doses per kilo of body weight were required in the dog to produce the lesion that quite regularly results from smaller doses in the rabbit. A dose of uranium nitrate in a dog three and a half times as large as the one commonly employed in the rabbit seemed to produce no more extensive lesion. This indicates that the dog's kidney is distinctly more resistant to the acute action of uranium nitrate than is the rabbit's kidney. The results of the various experiments are given in the following protocols:

PROTOCOLS

ANIMAL 489.—Female spaniel dog. Weight, 8.3 K. Caged June 13, 1912. Urine negative. June 16 received subcutaneously 3.3 mg. uranium nitrate per kilo of body weight. Killed June 17.

Kidney showed here and there deep in the medulla a single tubule or group of straight or convoluted tubules, with moderate necrosis and beginning cast formation. The glomeruli showed no lesion.

ANIMAL 484.—Mongrel dog. Weight, 12 K. Caged May 27, 1912. Urine negative. May 28 and 29 received subcutaneously uranium nitrate 3.3 mg. per K. of body weight. Killed May 30.

Kidney showed very slight fatty and hyaline degeneration of the convoluted tubules, with more extensive necrosis of groups of convoluted and straight tubules than in Animal 489. Glomeruli showed no lesion.

ANIMAL 450.—Brindle mongrel dog. Weight, 8.7 K. Caged March 10, 1912. Urine negative. March 13, 14, 15, received subcutaneously 3.3 mg. uranium nitrate per K. of body weight. Killed March 15.

Kidney showed moderate degree of necrosis and hyaline degeneration of the convoluted tubules, with more extensive necrosis of the collecting tubules. Many casts. A few glomeruli showed very small hyaline droplets in the glomerular tufts, for the most part in the wall of the capillary.

ANIMAL 474.—Mongrel dog. Weight, 8.3 K. Caged May 8, 1912. Urine negative. May 12, 13, 14, received subcutaneously 5 mg. uranium nitrate per K. of body weight. Killed May 15.

Kidney showed degenerative and necrotic lesions more marked in degree than in Animal 450, but the glomeruli appeared normal.

ANIMAL 281.—Mongrel dog. Weight, 9 K. October 16 received subcutaneously 7 mg. uranium nitrate per K. body weight. Killed October 23.

Kidney showed marked necrosis of the convoluted tubules and straight tubules throughout the cortex. Many casts. Many of the glomeruli showed fine hyaline droplets, for the most part, but not entirely, in the walls of the capillaries.

ANIMAL 332.—Coach dog. Weight, 12.7 K. Caged Oct. 2, 1911. Urine negative. October 5 received subcutaneously 5 mg. uranium nitrate per K. body weight. This produced albuminuria and casts, reaching the maximum October 10, gradually decreasing and disappearing. November 4, weight 11 K. November 4, 5, 6, received subcutaneously 7 mg. uranium nitrate per K. body weight. Killed November 6.

Kidney showed moderate necrosis of the epithelium of the tubules. Glomeruli showed no lesion.

ANIMAL 374.—Collie dog. Weight, 14.5 K. Caged Jan. 2, 1912. Urine negative. January 23, 24, 25, 26, received subcutaneously 7 mg. uranium nitrate per K. body weight. Killed January 27.

Kidney showed marked necrosis of straight and convoluted tubules, not quite so marked as in Animal 375. Several glomeruli showed hyalin droplets, but these were not confined to the wall of the capillary.

ANIMAL 375.—Bull dog. Weight, 16 K. Caged Jan. 23, 1912. Urine negative. January 24, 25, 26, received subcutaneously 8 mg. uranium nitrate per K. of body weight. Killed January 27.

Kidney showed marked necrosis of tubular epithelium. Glomeruli showed many hyalin droplets, but few of these were definitely confined to the wall of the capillary.

ANIMAL 449.—Black and white mongrel female dog. Weight, 15.4 K. Caged March 10, 1912. Urine negative. March 13, 14, 15, received subcutaneously 12 mg. uranium nitrate per K. body weight. Killed March 16.

Kidney showed marked necrosis of tubular epithelium, though probably no more extensive lesion than shown by Animals 374 and 375. In the glomeruli hyalin droplets were more common than in the preceding animals, and occurred both in and outside of the walls of the capillaries.

In all of these animals uranium nitrate produced in the urine the signs of acute nephritis.

CONCLUSIONS

The dog's kidney seems distinctly more resistant to the action of uranium nitrate than the rabbit's in so far as the production of anatomical changes is concerned. The glomerular lesion with the formation of hyaline droplets in the wall of the capillary tuft is produced with great difficulty in the dog.

Carney Hospital.

PARATYPHOID FEVER: A SEROLOGIC STUDY IN RELATION TO THE EPIDEMIOLOGY*

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During the course of epidemiologic investigations of typhoid fever the importance of the variability of clinical manifestations, the frequent failure of physicians to establish a diagnosis satisfactory to themselves, and the large percentage of cases in which positive agglutination reactions with the *Bacillus typhosus* were absent, particularly in water-borne epidemics, led to the observations herewith presented. It is the purpose of this paper to offer as a contribution the facts developed by serologic studies in certain epidemics to which I was assigned in charge under the direction of Dr. Samuel G. Dixon, Pennsylvania State Commissioner of Health.

GENERAL CONSIDERATIONS

By means of careful observations of the biochemistry and the agglutination properties of micro-organisms of the typho-colon type, it has been found that certain forms have characteristics which group them together; further, that their pathogenicity bears a somewhat definite relation to the grouping. For the purpose of study and in the light of present information, the classification based on the suggestion of Park[1] is used; in the order of pathogenicity it is as follows:

1. Typhoid group.
2. Paratyphoid group.
3. Dysentery group.
4. Colon group.

It is apparent from the literature that has accumulated since the first description of infections which are now known as paratyphoid fever that this disease is an unquestioned etiological entity. The bacilli—*B. paratyphosus A* and *B. paratyphosus B*—described as causing this pathological process have been divided into two types because of differences in cultural and agglutination phenomena. In the group to which they have been assigned, there are numerous other forms, the more important of which are *B. paracoli, B. alcaligenes, B. of hog cholera, B. suisepticus, B. enteritidis* (Gaertner), *B. of mouse typhoid, B. of calves' diarrhea* and *B. psittacosis.* To this date there is evidence that only certain members

* From the Pennsylvania State Department of Health.
* Submitted for publication March 3, 1913.
1. Park: Pathogenic Bacteria, 1910.

are pathogenic to man, and it is with these that we are particularly interested.

The clinical course of unreported cases has been considered indistinguishable from that of typhoid fever. It is not possible to present the results of clinical study in this contribution. There are many other features which remain to be clearly defined, such as the sources of infection, the methods of transmission, the association of the micro-organisms with the *B. typhosus* and the relation of clinical types to atypical forms of typhoid fever; it is desirable to know in what proportional relationship typhoid fever and paratyphoid fever and infections by other members of the typho-colon group may occur. It is also necessary for the application of preventive and therapeutic measures to know of what value anti-typhoid vaccine may be in cases of so-called mixed infections of this class, and, as to whether or not it would be advisable to use a polyvalent vaccine for the prevention of this group infection. These problems have, in part, been studied, and certain features will be considered in this article; others we hope to make the basis of future contributions.

The justification for separating paratyphoid fever from typhoid fever has been much discussed. In many epidemics of typhoid fever, particularly those in which the infection is water-borne, the earlier cases are atypical in onset and clinical course, and it is often difficult to convince the general practitioner that he is dealing with a disease which is bacillary in origin and which needs the same supervision as a typical case of typhoid fever.

My experience indicates that relatively few cases are diagnosed as typhoid fever. There is no doubt that many diagnoses of "catarrhal fever," "gastric fever," "grip," gastro-enteritis, abdominal influenza and others are made for patients suffering with a true *B. typhosus* infection, but far more frequently with infections by some pathogenic member of the paratyphoid group.

Each unreported case, escaping sanitary control, is the potential source of an epidemic and each case incorrectly diagnosed is inimical to the health of those acting as nurse and often as the household cook. In only one territory, that of Hawaii,[2] and in only one state, namely, Washington,[3] is it specifically required that the disease, as a clinical entity, shall be reported to the health authorities. In Pennsylvania, under the power granted to the Commissioner of Health, the latter may, with approval of the Advisory Board, declare a disease reportable. Such action has been taken during three epidemics which were under my immediate charge.

2. U. S. Pub. Health Bull., No. 45.
3. Rules and Regulations Washington State Board of Health, 1912.

In speaking of early cases, it is not meant that the *incubation* period of paratyphoid fever is not as long as that of typhoid fever; we have some evidence that the period for true infections by *B. paratyphosus A* and *B. paratyphosus B* is the same as of *B. typhosus*. Rolly,[4] in his studies of the so-called gastric form of paratyphoid fever, states that one of the peculiarities of paratyphoid fever is the insignificant incubation time. However, his observations were made on some 250 individuals whose infection was said to be due to *B. typhosus B*, and, as will be noted later, there is some probability that this was really a *B. enteritidis* infection, in which the incubation period is doubtless shorter than in true paratyphoid infections. There is, however, a shorter *prodromal* period for paratyphoid infections; the onset is more abrupt and the clinical expression of invasion and multiplication is more varied. The differentiation should be sharply made between the incubation period and the prodromal period.

Variations in clinical form lead to resemblances to many other types of infection. It is not desirable to discuss such differences in this paper, but in order to emphasize the need for a careful clinical and bacteriological diagnosis, the disease, *paratyphoid fever,* may be said to present one of four general types:

1. That closely resembling typhoid fever; this is most often due to *B. paratyphosus A*.

2. That closely resembling influenza of the abdominal type. This may be due to *B. paratyphosus A* or *B* or to mixed infections including these and *B. typhosus*, as well as *B para coli* or *B. alcaligenes*.

3. That suggesting a general gastro-enteric inflammation with nausea and vomiting. This is often diagnosed as ptomain poisoning and is due usually to one of the lower group members of the paratyphoid group.

4. That closely resembling dysentery, in which, however, *B. dysenteriae* is not found but most often *B. enteritidis* (Gaertner) is recovered from the feces and the agglutination reaction confirms the findings. According to the literature *B. paratyphosus B* is almost as frequently the offending micro-organism. This type, too, is often diagnosed as ptomain poisoning and almost as frequently "cholera morbus."

SOURCE

In considering the source of infection it is often difficult to limit the discussion to the true paratyphoid forms. There is no doubt from many observations which have been recorded that the various members of the paratyphoid group come from the intestinal tracts of domestic animals.

B. paratyphosus A.—This micro-organism in water-supplies was first isolated and identified from the spring water of an Italian village by Paladino-Blandino[5] in 1903. However, Schottmüller[6] ascribes six cases

4. Rolly, Fr.: München. med. Wchnschr., 1911, p. 559.

5. Paladino-Blandini: Ann. d'ig. sper., 1903, xv, 159.

6. Schottmüller: Deutsch. med. Wchnschr., 1900, p. 511; Ztschr. f. Hyg. u. Infectionskrankh., 1901, Bd. xxxvi; München. med. Wchnschr., 1902, No. 38.

reported by him up to 1901 to transmission by water. May,[7] in 1909, was successful in isolating the same form from running water which was used as the source of domestic supply. There are no other records of its recovery from drinking supplies.

Baermann and Eckersdorff,[8] while studying eight cases, isolated cultures from the feces. Harvey,[9] working alone, recovered the organism from the blood of eleven patients and many times from the feces, and later, working with Grattan[10] in the study of two small outbreaks, recovered the organism from the blood of seven patients and traced the source of infection to a cook who had suffered with cholecystitis several months previously, and whose blood showed a positive agglutination with the organism recovered from the other patients. Purjesz[11] isolated cultures from both feces and urine, and Aoki[12] recovered the organism in almost pure culture from an abdominal abscess and the feces. This patient was shown to be a carrier case and is so discussed by them. Monnier and Riberau[13] report an interesting recovery with autopsy, showing a positive agglutination for *B. paratyphosus A* in a dilution of 1 to 200, negative to *B. paratyphosus B*. The organism was recovered from the blood-stream, and on autopsy, was found in the spleen, liver and kidneys. However, isomutoric tests were not made in this instance.

B. Paratyphosus B.—The identification of *B. paratyphosus B* does not seem, to the present time, to be distinctly certain, and, in the review of the literature as presented, note is not made of the failure to differentiate sharply between this organism and *B. enteritidis* (Gaertner). The recent studies by Bainbridge would indicate that the particular strain of *B. paratyphosus B* recovered from cases of meat poisoning is entirely different from the true *B. paratyphosus B* referred to in the studies of this article. Bainbridge believes that all cases of meat poisoning are really infections by *B. enteritidis* (Gaertner).

B. paratyphosus B was first described by Achard and Bensuade[14] in 1896, and has been frequently recovered from the blood and dejecta of those ill with the disease. The first epidemic reported in the literature occurred in Saarbrucken,[6] Germany, in 1902, during which the bacillus was recovered from the feces in eighteen cases, and from the blood in four cases. Since that date many studies on organisms recovered from

7. May: Jour. Roy. Inst. of Pub. Health, 1909, xvii, 551.
8. Baermann and Eckersdorff: Berl. klin. Wchnschr., 1909, No. 40.
9. Harvey: Jour. Roy. Army Med. Corps, 1910, xiv, No. 4.
10. Grattan and Harvey: Jour. Roy. Army Med. Corps, 1911, xvi, No. 1.
11. Purjesz: Wien. klin. Wchnschr., 1910, No. 36, p. 1284.
12. Aoki: Centrabl. f. Bakteriol., 1 Orig. 1910, lvi, 110.
13. Monnier and Riberau: Compt. rend. Soc. de biol., 1910, lxix, 151.
14. Achard and Bensaude: Bull. et mém. Soc. méd. d. hôp. de Paris, 1896, xiii, 820.

water-supplies have been made. Gaehtgens[15] reports its isolation on three different occasions from a public water-supply, the source of which was a stream supplying an Alsatian municipality. In another instance, he recovered both *B. paratyphosus B* and *B. alcaligenes* from a fountain-supply.

Epidemics from ingestion of infected ice cream have been reported. Prigge and Sachs-Muke[16] studied thirteen cases in one outbreak. Liebetrau[17] reported an outbreak of nineteen cases of which three patients died; a nurse living in the home of a confectioner had nursed a paratyphoid patient. Both the nurse and the confectioner's helper showed a positive agglutination with *B. paratyphosus B,* and the epidemic followed the ingestion of ice cream manufactured by the helper.

Popps[18] reports paratyphosus B fever following the ingestion of spoiled oysters, the diagnosis being based on positive agglutination with *B. typhosus* and *B. paratyphosus B*; however, no other studies were made and the organism was not recovered from the blood or feces. König[19] studied four patients who, while at a convention, ate raw ham and whose blood gave a positive reaction with *B. paratyphosus B.* He later isolated the same organism from the ham. Friedrichs and Gardiewski[20] recovered a paratyphoid-like organism from tripe. It was not definitely identified. Rommeler[21] recovered the organism from pudding, sausage and tripe. Prigge and Sachs-Muke,[16] in another instance, studied sixteen cases of paratyphoid fever, apparently infected by roast swine. However, there were no bacteriological studies. Zimmerman[22] recovered *B. paratyphosus B* from one patient infected from eating the meat of a previously sick goose, and also an epidemic of sixteen cases from eating the meat of three calves which had previously suffered from calves' diarrhea. Heinemann[23] and Neumann[24] studied forty-seven cases in Cassel and found that only those who had eaten meat from a certain butcher shop gave a positive agglutination reaction with *B. paratyphosus B.* Rolly[4] duplicated this experience in a study of 250 patients, all of whom suffered from a gastric form. Neumann[24] reports its recovery from the blood, and Jensen and Kock[25] isolated a typical form from the bone sinus of an old abscess.

15. Gaehtgens: Arb. a. d. k. Gsndhtsamte, 1909, xxx, Part 3, p. 610.
16. Prigge and Sachs-Muke: Klin. Jahrb., 1909, xxi.
17. Liebetrau: Ztschr. f. med. Beamte., 1910, No. 2.
18. Popps, Fritz: München. med. Wchnschr., 1910, p. 584.
19. Konig: Centralbl. f. Bakteriol., Part 1, Orgl., 1909, l.
20. Friedrichs and Gardiewski: Centralbl. f. Bakteriol., Part 1, Orgl., 1909, li, 509.
21. Rommeler: Centralbl. f. Bakteriol., Part 1, Orgl., 1909, li, 501.
22. Zimmerman: Ztschr. f. med. Beamte., 1910, No. 3.
23. Heinemann: Ztschr. f. med. Beamte., 1911, Part 1, p. 2.
24. Neumann: Ztschr. f. med. Beamte., 1911, Part 1, p. 2.
25. Jensen and Kock: Deutsch. med. Wchnschr., 1910, p. 2196.

The evidence that the organism exists in the intestinal tracts of domestic animals has been demonstrated in at least three instances. Schmidt[26] and Conradi[27] recovered the organism from the feces of swine, and in 1911, Ruediger[28] recovered the organism from the feces of a dog. A private communication has been recently received which reports the recovery of the organism from the feces of the domestic cat. This organism was properly identified by all of the accepted criteria.

While there is some confusion as to the proper identification of *B. paratyphosus B,* in those instances in which bacteriological identification may be acceptable, organisms unquestionably belonging to the paratyphoid group have been recovered from the meat of calves, geese, swine, from sausage, ham, tripe, mince meat, Hamburger steak, from spoiled oysters, ice cream, public water-supplies and also from the intestinal discharges of certain domestic animals; they have been recovered from blood, feces, urine, abdominal and periosteal abscesses, ovarian carcinoma,[29] in pyelonephritis associated with urinary calculi,[30] and on autopsy, from the liver, spleen and kidneys of human beings. With this extraordinary array of infectious foci it is not to be regarded as curious that the cause of atypical forms of typhoid fever should be regarded by many writers as ubiquitous.[31]

FREQUENCY

The relative frequency of paratyphoid infections has been studied without definite conclusions. Gwyn's[32] case was the only one of 265 which did not react positively with *B. typhosus.* Schottmüller[6] and Kurth,[33] in their series of cases, found twelve which were negative to *B. typhosus,* but reacted positively to *B. paratyphosus.* Johnston[34] studied four cases occurring during an epidemic of 194 cases of typhoid fever. Hewlett[35] found one in twenty-six cases. Hühnermann,[36] at Saarbrucken studied thirty-eight cases of paratyphoid infection and found no cases of typhoid infection. Conradi[37] established a diagnosis of paratyphoid fever in twenty-nine of 250 cases, otherwise diagnosed as typhoid fever.

26. Schmidt: München. med. Wchnschr., 1911, p. 563.
27. Conradi: Klin. Jahrb., 1909., xxi, Part 2.
28. Ruediger: Bull. Manila Med. Soc., June, 1911.
29. Seiffert: Med. klin. Berlin., March, 1912, viii, No. 9.
30. Spassokukozky: Wein. klin. Wchnschr., March, xxv, No. 13.
31. Hubener: Deutsch. med. Wchnschr., 1910, p. 70.
32. Gwyn: Bull. Johns Hopkins Hospital, 1898, p. 54.
33. Kurth: Deutsch. med. Wchnschr., 1901, p. 501.
34. Johnston: Am. Jour. Med. Sc., August, 1902.
35. Hewlett: Am. Jour. Med. Sc., August, 1902.
36. Hühnermann: Ztschr. f. Hyg. u. Infectionskrankh., 1902, p. 522.
37. Conradi: Deutsch. med. Wchnschr., xxx, No. 32; Ztschr. f. Hyg. u. Infectionskrankh., 1903, p. 141.

In 1905, Fox,[38] in his review of the literature, found that the reported cases at that time were approaching a hundred, the diagnosis of some being complete, while that of others was questionable. They were, in part sporadic, in part small epidemics, and he measures the frequency of occurrence by the cases of apparent typhoid fever presenting a negative Gruber-Widal reaction. These, according to the Johns Hopkins Hospital report, amounted to 2 per cent. of all cases, while Schottmüller's[6] first study of sixty-nine cases of apparent typhoid fever showed that 4 per cent. were paratyphoid infections. All cases studied were sporadic and were those regularly admitted to the hospital from various localities in which, so far as is stated, the disease was not epidemic.

Proescher and Roddy,[39] in 1910, in a review of the literature up to that date, attempted to separate reported known cases of paratyphoid A from paratyphoid B, and reported that in the Allegheny County General Hospital, during 1908-09 "over fifty cases of paratyphoid A, about 200 cases of typhoid, but not one cases of paratyphoid B, were diagnosed." According to this report, 20 per cent. of all apparent typhoids were paratyphoid A infections.

Criteria for the proper identification of *B. paratyphosus A* and *B* were followed in but few of the cases studied early (prior to the work of Fox). However, the measure of incidence of either *paratyphosus A* or *paratyphosus B* infections has not yet been determined, and its relative frequency will differ for every outbreak in which there is a mixed infection.

HISTORICAL

ISELIN.—While studying an outbreak of typhoid fever in Iselin, Indiana County, during February, 1911, the atypical clinical course of cases which had been tentatively diagnosed by local physicians as typhoid fever and Asiatic cholera led to a somewhat restricted study of the blood of nineteen patients.

At the time it was impossible to secure blood-cultures and the agglutinating properties of the serum of the nineteen patients were studied with four members of the typho-colon group, viz., *B. typhosus B. paratyphosus, B. paracoli* and *B. enteritidis* (Gaertner). Seven agglutinated the *B. typhosus* and twelve the *B. paratyphosus B,* and were negative to all other members of the group studied. The reactions were studied in but one dilution and time period, namely, 1-50 for one hour.

Through a misunderstanding, the feces were examined only for the cholera vibrio,[40] and for that reason no micro-organisms of the typho-colon group were recovered.

38. FOX: Univ. of Penn. Med. Bull., April, 1905.

39. Proescher and Roddy: THE ARCHIVES INT. MED., 1910, v, 263.

40. Hetsch: Klin. Jahrb., 1907, xvi, p. 187, has observed that cases of paratyphoid infection, accompanied with severe diarrhea, may be mistaken for Asiatic cholera.

The twelve reacting with *B. paratyphosus B* were, all but one, of the type diagnosed as "Asiatic cholera"; that one patient, instead of having diarrhea, showed marked constipation, subnormal temperature, prostration and death.

The mortality in this outbreak, in which there were clinically diagnosed twenty-two cases of typhoid fever and thirty-one of paratyphoid fever, showed that four, or 12.5 per cent., of the latter resulted fatally; while in ten, or 45 per cent. of the cases diagnosed as typhoid fever the patients did not recover. However, it should be pointed out that in the majority of cases in which death occurred and where typhoid fever had been diagnosed clinically, the patients had been referred to a hospital where no serologic studies were made, and there is a possibility of error in these figures. The four cases of paratyphoid fever which resulted fatally, had all been diagnosed serologically, as well as clinically.

BETHLEHEM.—During the subsidence of an epidemic of bacillary dysentery in Bethlehem during September, 1911, a series of eighteen cases were studied. The agglutination reaction of six of these was positive with *B. typhosus* only; one was positive with both *B. typhosus* and *B. paratyphosus B*, while eleven agglutinated *B. paratyphosus B* only. Negative reactions were obtained with *B. paratyphosus A*, *B. paracoli*, *B. enteritidis* (Gaertner), *B. suicida* and *B. alcaligenes*.

From the feces, in addition to the common forms, there were recovered *B. suicida*, *B. enteritidis* (Gaertner), *B. acidi lactici*, and two strains described by Kruse as *B. paradoxus* and *B. pseudotyphoid*. From the spring source of the public water-supply, which was the transmission agent causing the epidemic, *B. alcaligenes*, *B. suicida*, *B. acidi lactici*, *B. coli communior* and a dysentery form (*B. Rosen*) were recovered and identified in the usual way.

Two of the patients showing positive reactions to paratyphoid organisms died, a mortality of 16.6 per cent. There were 102 cases of typhoid fever reported during the last four months of the year in which the eighteen cases studied were included. The latter were the first to develop after an epidemic of dysentery[41] had passed its acme, and all showed atypical onsets and clinical courses. Subsequent cases infected at the same time gave a history of a longer prodromal stage and were typical *B. typhosus* infections.

BLANDON.—In an outbreak of thirty-five cases in Blandon, Berks County, during November and December, 1911, six atypical cases were studied. The blood of one reacted with *B. paratyphosus A* only, one with *B. typhosus*, *B. para coli* and *B. paratyphosus B*, and four with *B. typhosus* only. Of five micro-organisms recovered from the feces of

41. Hunt: Jour. Am. Med. Assn., 1912, lix, 919.

five cases, two proved to be *B. typhosus*, one was identified as *B. paratyphosus A*, one was *B. paratyphosus B* and one *B. enteritidis* (Gaertner).

COATESVILLE.—One Feb. 11, 1912, I was assigned to visit Coatesville, Chester County, for the purpose of establishing a diagnosis. Ten cases were studied and a tentative diagnosis of typhoid fever was made in three cases, and of paratyphoid fever in seven cases.

An investigation of the conditions in Coatesville showed that in the practice of nine physicians there were 131 cases in which a diagnosis had not been definite, many of the physicians, however, suspecting that they were dealing with an atypical type of typhoid fever; none of the cases had been reported. There are eighteen physicians in Coatesville, the 131 cases occurring in the practice of 50 per cent.

Facts which were known to the state department of health, in connection with evidence collected on the same date showed that it was a water-borne infection, and the definite source of pollution was found to be from human sewage, some of it from patients clinically diagnosed as having typhoid fever; in addition the water-supplies were found to be grossly polluted with sewage from domestic animals. The history of a case of meat poisoning or of paratyphoid fever could not be obtained at the polluting sources. The gross pollution of the public water-supply occurred between Jan. 19 and Jan. 23, 1912. The earliest date of onset was January 19; 334 cases were reported as typhoid fever to the local board of health, the mortality of which was thirty-five, or 10.5 per cent.; of those diagnosed serologically, the mortality was three, or 0.9 per cent., of these one reacted positively with *B. typhosus* only, one with *B. paratyphosus A* only, and one with *B. typhosus* and *B. paratyphosus A*.

Studies of the water, feces and blood of seventy-six patients were immediately undertaken, because of the many atypical cases which occurred.

The water examination showed the presence of large numbers of *B. paradoxus* (Kruse), *B. coli communis* and *B. cloacae*. No other members of the typho-colon group were recovered. The three types recovered were taken from samples obtained from the settling basin, the borough water mains and Newlin's Run, which was the polluted source of the epidemic.

No members of the typho-colon group were recovered from the few specimens of feces studied.

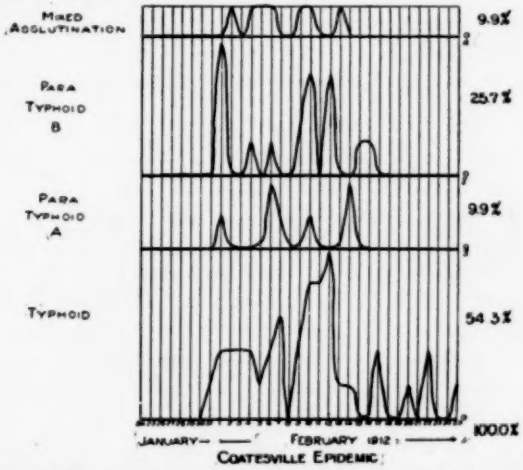
Blood-cultures were made, but with negative results. No case could be found which did not show a positive serum reaction. It is probable that the specific micro-organism had disappeared from the blood because of the presence of antibodies appearing coincident with the formation of agglutinins.

The seventy-six cases gave positive serum reactions in the following proportion:

54.5 per cent. were positive with *B. typhosus* only.
25.7 per cent. were positive with *B. paratyphosus B* only.
9.9 per cent. were positive with *B. paratyphosus A.* only.
9.9 per cent. were positive with more than one group, members as follows:
1.4 per cent. *B. typhosus* and *B. paratyphosus A.*
5.7 per cent. *B. typhosus* and *B. paratyphosus B.*
1.4 per cent. *B. paratyphosus A.* and *B. para-coli.*
1.4 per cent. *B. paratyphosus B.* and *B. para-coli.*

It should be stated that these patients were selected at random (with reference to atypical characteristics) and were not chosen from any one group or type. They represent cases with dates of onset from January 19 to February 25, covering as wide a range as possible.

The dates of onset of the seventy-six cases are plotted in the accompanying diagram.



Agglutination reactions in the sera of seventy-six patients showing the relation of positive reactions to dates of onsets of the disease.

It will be observed that the curves representing the dates of onset of each group coincide to a very remarkable degree and would seem to indicate that the incubation period for the various members of the groups studied is practically the same. However, the prodromal stage, in all cases showing a reaction with more than one organism, and those giving a reaction with *B. paratyphosus B* only, was short and atypical; the onsets were abrupt.

The serums of all cases, in addition to the above, were studied with *B. enteritidis* (Gaertner), *B. suicida, B. dysenteriae* (Shiga) and *B. Rosen.* The results were negative in every instance except as given in the table.

In order that we might have an accurate idea as to the specificity of the reactions noted in this table, the agglutination reaction for each laboratory organism used was studied with serums from supposedly healthy persons. There was no agglutination of any of the types studied in dilutions of 1-10, 1-20, 1-40, 1-80 and 1-100, except that in one person a slight tendence to clumping without loss of motility of *B. typhosus* occurred in dilutions of 1-10. All control cultures were decidedly motile after one hour's observation.

The organisms used were obtained from the following sources: *B. typhosus* from the Philadelphia Bureau of Health Laboratory; *B. paratyphosus A* from the Laboratory of Hygiene, University of Pennsylvania; *B. paratyphosus B* from the same source, but was obtained by them from Dr. Cushing of Boston; *B. paracoli* from Dr. Buxton, *B. dysenteriae* (Shiga) was obtained from the Laboratory of Hygiene, University of Pennsylvania; *B. enteritidis* (Gaertner) from the same laboratory; *B. spicida* was isolated from feces in the Chester dysentery[42] outbreak in 1910 by Herbert Fox and J. B. Rucker, Jr., while *B. Rosen* was isolated from Bethlehem[41] water and feces during September, 1911, by Rucker.

SUMMARY

To recapitulate: During the course of four water-borne epidemics of typhoid fever, including 509 cases, 117, or 22.9 per cent., presented atypical features, either during the onset or clinical course.

From the feces of eleven patients, in addition to the common inhabitants of the gastro-intestinal tract, there were recovered *B. suicida, B. enteritidis* (Gaertner), three times; *B. acidi lactici,* twice; *B. paradoxus* (Kruse), twice; *B. pseudotyphosus* (Kruse), once; *B. typhosus,* twice; *B. paratyphosus A,* once; *B. paratyphosus B,* once, and *B. cloacae,* once.

From the water (in one epidemic) were recovered *B. alcaligenes, B. suicida, B. acidi lactici, B. coli communior* and a dysentery organism identified as one described by Rosen in an epidemic of bacillary dysentery at Rotterdam.

The agglutination reactions of the blood-serums from the 117 patients were studied in 1-50 dilutions and gave the following results during a period of one hour or less:

Micro-Organisms.	Number.	Per cent.
B. typhosus only	54	46.15
B. paratyphosus A only	9	7.7
B. paratyphosus B only	47	40.2
B. typhosus A and *B paratyphosus*	1	0.86
B. typhosus and *B. paratyphosus B*	2	1.71
B. paratyphosus A and *B. paracoli*	2	1.71
B. paratyphosus B and *B. paracoli*	1	0.86
B. typhosus B, B. paratyphosus B and *B. paracoli*	1	0.86
	117	100.00

42. Report of Commissioner of Health, Pennsylvania, 1910.

In consideration of the foregoing, the following questions naturally suggest themselves:

1. What relation does a negative agglutination with *B. typhosus* bear to the presence of paratyphoid fever?

2. What is the influence of "group" agglutinins?

3. How nearly specific is the Gruber-Widal reaction as practiced in the usual hospital and private laboratory service?

The answer to the first question depends on a number of factors and includes a consideration of the second question. The proportion of negative reactions with *B. typhosus*, as has been previously noted, is very low (according to Fox,[38] 0.4 per cent. of proven cases); the basis for estimation, however, was from cases, all of which were collected from the literature, and included but one outbreak of any magnitude (Saarbrucken), the origin of which was found to be in meat. No water-borne epidemic of any size which included a study of atypical cases has been reported; however, as Jordan[43] suggests, both typhoid and paratyphoid fever may exist undifferentiated in the same epidemic, and the full extent of water infection for para cases is not fully recognized. The proportion of negative results in the Coatesville epidemic seems to confirm this suggestion, since 38.4 per cent. of cases were at all times negative with *B. typhosus*.

TIME OF APPEARANCE

Agglutinins in typhoid fever have been closely studied; they appear as early as the fifth day of disease, but may not appear until the fourth week, or even not until during the second month; various statements as to their time of appearance have been made by various observers. A compilation of these observations resulted as follows: In from 18 to 25 per cent. of cases they appeared during the first week (i. e., after the fifth day), 60 to 65 per cent. during the second week, 80 to 90 per cent. during the third, and upwards of 94 per cent. during the fourth and subsequent weeks. No observations are recorded as to the appearance of agglutinins peculiar to paratyphoid infections. However, there is no reason to believe that the same proportional relation to time of appearance would be different from that found in typhoid fever.

In the Coatesville epidemic, agglutinins reacting positively with the micro-organisms named in the following table were present on the days after onset as indicated:

Paratyphosus A

Days after onset first observed.	Number of cases.
5	1
9	2
12	1
13	2
23	4

43. Jordan: General Bacteriology, 1911.

Days after onset first observed.	Number of cases.
Paratyphosus B	
5	1
8	1
9	2
10	1
11	1
13	2
14	3
15	2
18	4
21	1
22	2
39	3
Unstated	1
B. typhosus and B. paratyphosus A	
5	1
12	1
B. typhosus and B. paratyphosus B	
17	1
18	2
19	1
24	4
B. paratyphosus A and B. paracoli	
13	1
B. paratyphosus B and B. paracoli	
14	1

It seems justifiable to assume that the time of appearance of agglutinins in paratyphoid does not differ from typhoid fever.

SPECIFICITY

It is possible that group agglutinins may account for some of the results obtained, but there are reasons for accepting the relative specificity of the reactions recorded. First, because the agglutination reaction of a laboratory strain of *B. typhosus* has been shown to be greater than for any related micro-organisms with which it has common or group agglutinins (such as *B. coli, B. enteritidis, B. paratyphosus B*); second, the power of a given serum to agglutinate *B. typhosus* in high dilutions practically always will be accompanied by the power to agglutinate the related bacilli in a much lower dilution; that is, the specific agglutinins in *B. typhosus* are usually *far* in excess of the non-specific or group agglutinins; and, third, group agglutinins are less likely to be present in normal serums. All studies reported in the literature have been made with immune serums.[1, 4, 38, 43, 44, 45] The variation of specific and group agglutinins has been noted, not only in bacteria biologically related, but

44. Hiss and Zinsser: Bacteriology, 1910.
45. Rimpau: Munchen. med. Wchnschr., 1909, p. 1843.
46. Wolff: Centrabl. f. Bakteriol., 1899, xxv.

also in different strains of the same organism.[1, 46] This variation, however, in view of the fact that specific agglutinins are always far in excess of group agglutinins should not detract from the specificity of these observations.

Jordan[45] points out the fact that the specific agglutinins produced by the two types of para-organisms, as those of *B. typhosus*, are entirely different. While Park[1] states that because of the lack of absolute specificity, the diagnosis of the type of infection or the absolute identification of the micro-organism can only be determined (through agglutination or bacteriolytic tests) with a certain degree of accuracy.

Various criteria for determining the specificity of the infecting micro-organism have been suggested.

Fox[38] states that "the best proof of the existence of a para-infection is the isolation of the bacterium, the saturation test, and, last, the bactericidal action."

Mayer[43] suggests the use of the Pfeiffer test (bactericidal) when there is a negative agglutination and atypical clinical course with recovery of *B. paratyphosus B* from the feces. Libram[47] emphasizes the value of the saturation test to differentiate infections by *B. typhosus* and *B. Gaertner*. Neuman[24] relied in the diagnosis of sixty cases on the recovery of a micro-organism from the blood and positive agglutination. Kallmeyer[48] reports one case in which he obtained a positive agglutination with *B. paratyphosus A* in 1-40 and 1-60 dilutions, and negative reaction with *B. typhosus*.

Grimm[49] states that the Gruber-Widal reaction in its mutual relations is specific in the early stage, but believes that a single examination often fails to fix the diagnosis between typhoid and paratyphoid infections because of atypical reactions caused by preformed agglutinins. He states that the occasional determination in high dilution in normal human serums for paratyphoid fever shows just as little as the recovery of the bacillus from the feces.

Hiss[44] decides that the specificity of reactions for practical purposes is not destroyed if proper dilution is carried out, as the degree of agglutinin formation is always higher for the specific bacterium than for allied micro-organisms. His diagnostic criteria would then depend on recovery of the bacillus and the study of immune serums.

Park[1] states that "the only reliable criteria for diagnosis" are negative reaction with *B. typhosus* in a dilution of not less than 1-40 with a positive reaction to a paratyphoid bacillus the agglutinability of which is known, or the recovery of a para form from the blood, urine or complicating inflammatory process.

47. Libram: Ztschr. f. Hyg. u. Infectionskrankh., 1909, lxiv, Part 3, p. 411.
48. Kallmeyer: St. Petersburg med. Wchnschr., 1909, No. 25.
49. Grimm: Centrabl. f. Bakteriol., Abt. 1, Orgl., 1910, liv, Part 4, p. 367.

From these varied opinions it is difficult to conclude what criteria are acceptable. As Jordan points out, the absorption of specific agglutinins with the homologous organism removes not only the specific, but also part or all of the group agglutinins, and that bacteria may even remove agglutinins which they have not produced and which do not visibly affect them. Hence, the use of Castellani's test may lead to wrong conclusions unless high enough dilutions can be used to exclude all action of group agglutinins. The use of this test to determine "mixed infections" may, for the same reasons, lead to false deductions.

There is reason to believe that the bactericidal power of serums bears no relation to the presence of agglutinins. This has been apparently carefully worked out by the use of Pfeiffer's test and by studies on the relationship between bacteriolysins and agglutinins.

The recovery and absolute identification of the causative micro-organism is of unquestionable value, especially when isolated from the blood-stream. That this can be done without elaborate technic is shown in the report of Schweinburg,[50] who, while examining 129 specimens of blood collected for the agglutination reaction in the usual way, made cultures with the following results:

Week of Disease	No. of Cases	Positive Culture	Per Cent.	Positive Agglutination	Per Cent.
1	67	65	97	57	84.9
2	40	32	80	36	90
3	10	5	50	8	80
After third	12	Neg.	..	Neg.	..

In four specimens the organism isolated was identified as *B. paratyphosus A;* in all others, *B. typhosus.* (The tabulation is mine.)

The recovery of *B. paratyphosus A* or *B* from the dejecta seems less conclusive[10] since a variety of non-pathogenic types are also recovered from the feces of a relatively large number of domestic animals and humans. Given the presence of distinct clinical phenomena and the appearance of agglutinins in the blood-serums, the recovery of an organism from the feces has the greater value.

LABORATORY USAGE

The answer to the third question is found in the consideration of the Coatesville tabulations. Practically but few laboratories do much more than study submitted specimens of blood-serums with *B. typhosus,* usually in low dilutions — 1-20 or 1-40. As a rule, however, the agglutination reaction of a laboratory strain of known agglutinability (as noted above) is greater than for related micro-organisms, and there is no opportunity for interagglutination of a lower group member confusing a positive

50. Schweinburg: Wein. klin. Wchnschr., 1910, No. 9.

diagnosis. It, however, makes no provision for a negative result in the possible instance of lower group infection. We are justified in assuming that the reactions as routinely practiced in hospital and other laboratories are only as specific as the presence of typhoid fever is in the cases examined, and hence there is additional reason for determining the reaction in negative cases with members of the paratyphoid group. During the past two years this work has been carried out in the laboratories of the Pennsylvania State Department of Health, but only in epidemic work.

CONCLUSIONS

These studies during the course of four epidemics indicate that we were dealing with unusual and widely varied types. It is probable that each type was specific, if Park's criteria are acceptable. The serums, collected at varying periods of from seven to thirty-seven days, studied in dilutions of 1-50 with known organisms for at least one hour, with as many as three studies in several cases, reacted only with the types recorded; hence the specificity of the infecting bacilli as determined by these tests is probably accurate.

Many observers express the opinion that the differentiation of paratyphoid infections from typhoid fever is of importance only from an etiological standpoint. It would appear that the differentiation has a much greater significance. The rôle in transmission of the undiagnosed cases (as in the bacillus carrier) has not been statistically determined. If in fifty-nine of 509 cases, or 11.5 per cent., of all patients, there is a constant negative Gruber-Widal, it is of greatest importance to show the relation of this to some other and just as serious infection.

The doubt of many practitioners of the existence of atypical cases is more prevalent than can be easily understood and is very apparent to the sanitarian, often to an uncomfortable extent, since he must secure results through the *modus operandi* of legal procedure, or, at least of persuasion, and the difficulties in diagnosis are reflected in the difficulties to secure proper reporting and supervision.

The use of the agglutination reaction of the normal serum is the only laboratory method available to the vast majority of general practitioners. The reliance on this point is practically absolute among the large majority of physicians, and it is not curious when one refers to clinical literature and teachings.

The results seem to indicate the following:

1. The great variation of types of infecting micro-organisms.

2. Paratyphoid infections are probably endemic in Pennsylvania.

3. The importance of differentiating infections by types of the typho-colon groups.

4. The importance of the infection of water-supplies from the fecal discharges of domestic animals as well as of human beings.

5. The suggestion that a mixed vaccine is of more importance than one of *B. typhosus* only.

I desire to express my very great appreciation to Dr. Samuel G. Dixon, Commissioner of Health, for this opportunity; to Dr. Herbert Fox, J. B. Rucker, Jr., and Alexander Garcia for their laborious work, and to many physicians in Iselin, Bethlehem and Coatesville, who made it possible to study their patients.

THE RESISTANCE OF RETICULATED ERYTHROCYTES*

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Ever since the recognition of the many interesting phenomena revealed by the application of the method of vital staining to the erythrocyte, hematologists have been endeavoring to derive from these findings facts of practical importance. As each discovery was announced some significance was attached to it, and the hope was raised that thereby some light was to be shed on the etiology of blood-diseases or on the vexed question of destruction and regeneration of the blood. It is now almost twenty years since the erythrocyte began to be studied by this means, and during this time almost twenty varieties of intra-corpuscular phenomena have been demonstrated and a most voluminous literature has collected. The majority of these findings, however, have not as yet been shown to be of any significance, many have been explained as merely artefacts and only a few are now considered worthy of mention in the newer works on hematology. Also, unfortunately, much of the work has been described without correlation with previous reports, and this has led in some instances to the same finding being credited to different discoverers in different countries, with, therefore, more than one name attached to it and often with the application of more than one significance. It is remarkable, however, how little discussion of this subject appears in English or American medical literature, whereas French, German and Italian articles are numerous. For these reasons the main points of the general subject are here reviewed somewhat in detail.

The one type of erythrocytic alteration demonstrable by vital staining which has been universally accepted as of importance is known variously as "skeined" or reticulated erythrocytes (*Hematies granuleuses* of the French), the basic staining substance of which is often spoken of as the Rosin-Demel substance, after two of the early workers in this field, or as *Fadenförmige substanz* or *Filarmasse* of the Germans, *Substantia granulo-filamentosa*, etc. Briefly, it may be described as an intra-erythrocytic phenomenon, demonstrable only by vital staining with a basic dye, occurring in a small percentage (0.5 per cent.) of the erythrocytes of normal blood and in much larger percentages in many pathologic conditions. Its distinctive structure is suggested by its various names and is quite variable; sometimes being only a few granules connected by fine threads,

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at other times a net-work of threads with many granules, and again a thick ball-like tangle of threads. Its appearance is always characteristic and it cannot be confused with any of the other basophilic changes in the erythrocytes. It may be demonstrated either in wet vitally-stained preparations or in vitally-stained preparations which have later been fixed and which may be counterstained with Giemsa's or Wright's stain.

Although at first some investigators believed that this basophilic substance of the reticulated erythrocytes was an indication of disease of the cell and others that it was due to beginning degeneration, the more recent opinion is almost unanimous that these skeins are only to be found in young erythrocytes and that an increase in the number of reticulated cells is evidence of unusual activity in blood production. It is also generally accepted that they are analogous to the polychromatophilic cells of fixed preparations, but their relation to the common basic granulations of fixed preparations is still a much mooted point.

A simple enumeration of the reticulated cells in various types of disease has been repeatedly made and with quite uniform findings, but with results of little practical significance. If it is true that these cells represent young cells and that their number is an index of blood production, several interesting problems present themselves. The two most obvious ones to which this method might be applied have, however, been but little studied. In the first place the percentage of skeined forms might be employed as a means of estimating the therapeutic activity of hematinic medication or other therapeutic measures and this we are at present undertaking in connection with the use of benzol in leukemia. The second possible application is to the problems concerned with the attempt to interpret, as characteristic of young cells, the other alterations in the properties of the erythrocytes associated with the increase of reticulated forms. Of these alterations the one in which we have been interested is the increased resistance of the erythrocytes, under given conditions, to sodium chlorid hemolysis and the relation of this increased resistance to the percentage of reticulated forms present. In other words, does the reticulated erythrocytes show increased or decreased fragility as compared with the unreticulated cells of the same blood, and does this relationship exist in all conditions of altered hemolysis? This problem is also concerned with the degree of hemolysis produced by the gradations between the maximum and minimum hemolysis and the type of cells affected. By maximum resistance is meant that concentration of sodium chlorid in which complete hemolysis occurs, while by minimum resistance is meant the concentration of sodium chlorid solution in which the least discoloration of the fluid is preceptible. What degree of hemolysis is produced by the solutions of intermediate strength is known only in an indirect way through the percentage estimation of the hemoglobin con-

tent of the hemolytic solution, as compared with the content of a similar amount of solution containing the products of complete hemolysis of the same amount of corpuscles. This method was introduced by Theobald Smith and Brown[1] and later used by Gay.[2]

A number of authors have assumed from the coincident alteration in resistance and increase in reticulated forms that the former was dependent on the latter. Such assumptions have been made in almost all conditions that show both these alterations from the normal, and so theoretically has this been done that both increase and decrease of erythrocytic fragility have been attributed to an increased presence of young skeined cells.

The increase of skeined forms in the newly-born infant is very marked and may reach 20 per cent. Cathala and Daunay[3] have attempted to show that the physiological increase of skeins seen in the embryo and during the first few days of life is the explanation of the decrease of resistance of red cells observed by them at this time, but they conclude that the two findings do not alter in proportion to each other. On the other hand, some observers have noted an increased resistance of the fetal erythrocytes which continues a short time after birth.

The list of pathological conditions in which an increased number of reticulated forms has been described is long, but many of these conditions can be grouped together if the underlying causative anemia is recognized. Briefly, it may be said that all anemias, except those which have become aplastic through chronicity or lack of regenerative power of the bone-marrow, show an increased percentage of skeined forms. Also it may be said that in almost every anemia there is an alteration in the resistance of the erythrocytes to sodium chlorid hemolysis and this alteration is almost constantly an increase of resistance more or less proportionate to the degree of anemia. Here again authors have assumed a relationship between the two phenomena, and especially so in secondary anemia, where both the resistance and the number of reticulated cells are, as a rule, increased. Pernicious anemia shows a similar state of affairs, and in this disease the presence of skeins is of importance since their disappearance is a strong argument, as has been emphasized by Vaquez[4] for the diagnosis of aplastic change of the bone-marrow.

1. Smith, Theobald, and Brown, H. R.: The Resistance of the Red Blood Corpuscles of the Horse to Salt Solutions of Different Tonicities before and after Repeated Withdrawals of Blood. Jour. Med. Research, 1906, xv, 425.

2. Gay, Frederick P.: The Function of Tonicity in Human Isohemagglutination. Jour. Med. Research, 1907-8, xvii, 321.

3. Cathala, V., and Daunay, R.: Les hématies granuleuses, la résistance globulaire à la naissance et pendant les premiers jours. Compt. Soc. de biol., 1908, lxiv, 801.

4. Vaquez, M. H.: Anemie et rénovation sanguine. Bull. et mém. Soc. méd. d. hôp. de Paris, 1907, xxiv, 1532.

The history of the development of this point of view is interesting. In a case reported by Widal, Abrami and Brulé,[5] the patient suffered from an azotemic nephritis with intense anemia which resembled the aplastic type, but many skeins were present in the blood and no myelocytes; the fragility was normal. At autopsy the bone-marrow gave every appearance of activity. At the same time these investigators studied the effect of bleeding in animals and produced thereby an anemia with cells showing an increased number of skeins and an increased resistance to hemolysis. At this result they were greatly surprised, for at the time the French school believed the *"hematies granuleuses"* to be diseased corpuscles and to have a lowered resistance. They also, in another series[6] of experiments, produced an hemolytic anemia and observed that the resistance became lowered before any increase of reticulated cells appeared, and that the two phenomena did not coincide; also, they observed that similar conditions may be seen in human cases; that is, that either phenomenon may occur without the other. The opinion of the French school that the reticulated forms are associated with decreased resistance to hemolysis was due to the observations of Chauffard and Fiessinger, who, while looking for some disease of the erythrocyte that would help to explain the increased fragility seen in congenital hemolytic jaundice, found that the blood in such cases showed an enormous number of *hematies granuleuses.* To these they attributed the decreased resistance and considered such cells as diseased corpuscles. Their finding has been frequently confirmed, but not their interpretation. No one now believes the reticulation of the erythrocytes to be an evidence of cellular disease or of autolysis as was believed by Feuillé. It is in these cases of so-called congenital or familial hemolytic icterus that the highest counts of skeined corpuscles are seen, sometimes as many as 65 per cent. of the erythrocytes showing this phenomenon.

Heinz,[7] in 1890, was the first to observe changes in the erythrocytes following the experimental injection of pyrodin, or phenyl-acetyl-hydrazin, a substance closely related to phenylhydrazine which is now more frequently used in laboratory work. The alterations which he observed and described are generally called, after him, the "Heinz bodies." These bodies he demonstrated by vital staining with a fluid prepared by saturating a 0.6 per cent. sodium chlorid solution with methyl violet. Their appearance is that of small blue staining knobs at the periphery of the erythrocytes, one or two to a cell, and of about the

5. Widal, F., Abrami, P., and Brulé, M.: Anémie grave mortelle chez une brightique azotémique. Bull. et mém. Soc. méd. d. hôp. de Paris, 1907, xxiv, 1427.

6. Widal, F., Abrami, P., and Brulé, M.: Pluralité d'origine des ictères hémolytiques. Bull. et mém. d. Soc. méd. d. hôp. de Paris, 1907, xxiv, 1354.

7. Heinz, R.: Morphologische Veränderungen der rothen Blutkörperchen durch Gifte. Virchows Arch. f. path. Anat., 1890, cxxii, 112.

size of a blood-platelet which, indeed, many authors have believed them to be. Heinz, however, considered them to be particles of necrotic protoplasm, and this view is most generally accepted. They have been found also to correspond to the "inclusion bodies" of Ehrlich,[8] which are seen in fixed preparations. Many further investigations were carried out in experimental phenylhydrazine poisoning, and it was discovered that in addition to the production of Heinz bodies by this blood-poison there also occurred a great increase in the number of reticulated cells. Later, Morawitz and Pratt[9] announced that in experimental phenylhydrazine poisoning in the rabbit there occurred also a very marked increase of the resistance of the erythrocytes. This increase is so great that after the fourth daily injection of 0.01 gm. phenylhydrazin chlorid the erythrocytes are not completely hemolyzed even by distilled water. This increase of resistance holds also for all types of hemolysins and is associated with the intense anemia which results from the injections of the poisons. A further phenomenon in the blood in such toxic anemias was described by Itami and Pratt[10] as an increase of stroma which they found went parallel to the increase of resistance. Rosenthal,[11] however, found no such relationship, and believes that the increase of stroma should perhaps be considered as analogous to cloudy swelling. Suzuki[12] made an exhaustive study of the blood in phenylhydrazine-poisoned animals, and concludes that the heightened resistance is due not so much to a diffuse *"pachydermie"* as to the formation and presence of Heinz bodies. Cells containing Heinz bodies he believes to be highly resistant in all test solutions and it is these cells which become larger, more voluminous and more irregularly shaped the longer the poisoning lasts. He came to this general conclusion as a result of studying the sediment of blood partially hemolyzed with 0.6 per cent. saponin. By staining this sediment he obtained little else than an enormous mass of Heinz bodies with only an occasional shadow corpuscle. In the sediment of a 10 per cent. saponin solution he found nothing but well-preserved Heinz bodies. In a similar way, Hartwick[13] prepared his so-called pure culture of Heinz bodies for chemical study. It does not seem probable that the persistence of these

8. Ehrlich, P.: Kongress für innere Medizin, Leipzig, 1892.

9. Morawitz, P., and Pratt, J.: Einige Beobachtungen bei experimentellen Anämien. Münch. med. Wchnschr., 1908, lv, 1817.

10. Itami, S., and Pratt, J.: Ueber Veränderungen der Resistenz und der Stromata roter Blutkorperchen bei experimentellen Anämien. Biochem. Ztschr., 1909, xviii, 302.

11. Rosenthal, F.: Die sogenannte Pachydermie der Erythrozyten bei Phenylhydrazinämie. Folia haemätol., 1910, Arch., x, 253.

12. Suzuki, T.: Weitere Beiträge zur Kenntnis der Erythrozytenveränderungen bei Pyrodinvergiftung. Folia haematol., 1912, Arch., xiii, 225.

13. Hartwich, W.: Weitere Beitrage zur Kenntnis der Heinzschen Vergiftungskörper (Ehrlichsche hämeglobinamische Innenkörper). Folia haematol., 1912, Arch., xiii, 257.

bodies explains the apparent increased resistance of the erythrocytes since the Heinz body is but a small portion of the cell and its persistence would in no way interfere with the hemolysis of the remainder of the erythrocyte.

To summarize: In experimental work, a relationship has been assumed between the increased resistance and the increase of intra-erythrocytic phenomena, just as has been done in clinical types of anemia; but this has never been proved.

Direct attempts have been made in several ways to estimate how many and which cells are hemolyzed by the various degrees of hemolysis. Snapper,[14] apparently unfamiliar with the work of Smith and Brown,[1] and Gay,[2] attempted to reach some conclusion by estimating the amount of hemoglobin in solution in the various percentages of salt solution and reached the conclusion, on what grounds it is difficult to see, that the increased resistance in experimental anemia is due to young erythrocytes, and that it is proportionate to the degree of regeneration. In a rough way he attempts to draw numerical relations, but fails to reach any positive finding. Smith and Brown concluded that the facts concerning hemolysis do not permit the hypothesis that the younger erythrocytes, as a group, are either less or more resistant than the older cells, and that the theory most in harmony with the facts disavows any relationship between age of corpuscles and resistance to salt solutions. Nor could they find any close correspondence between size of corpuscles and the resistance.

In a manner similar to that employed by Suzuki in his studies of Heinz bodies, a few investigations have been made of the sediment, in tubes of partial hemolysis, to determine the presence and percentage of reticulated forms. Among the first reports of this nature was that of Chauffard and Fiessinger,[15] who, in 1907, studied the reticulated corpuscles at various stages of hemolysis and, contrary to their expectations, did not find them disappearing more quickly or more readily than the normal erythrocytes; in fact, the skeined forms seemed to persist longer. Fiessinger[16] later confirmed these findings. Sabrazes and Leuret[17] also made observations in a similar manner — on the blood of icteric infants which sometimes contained as many as 18 per cent. of skeined forms — and found that the reticulated cells did show increased resistance.

14. Snapper, J.: Vergleichende Untersuchungen über junge und alte rote Blutkörperchen. Biochem Ztschr., 1912, xliii, 256.

15. Chauffard, A., and Fiessinger, N.: Recherches experimentales sur les rapports entre l'hémolyse et les hématies granuleuses. Bull. et mém. Soc. méd. d. Hôp. de Paris, 1907, xxiv, 1367.

16. Fiessinger N.: Congrés de méd. de Lyon, October, 1911.

17. Sabrazes, J., and Leuret, E.: Hématies granuleuses et polychromatophilie dans l'ictere des nouveau-nés. Compt. rend. Soc. de biol., 1908, lxiv, 423.

The most careful work in this respect has been done by Ravenna,[18] who estimated the fragility according to Hamburger's technic and after staining the sediment made careful counts of the percentage of skeined forms found. These counts showed a marked increase over the percentage found in the unhemolyzed blood. The counts were made both from fixed smears of the sediment after staining and from fresh-stained preparations by the use of a counting pipet and chamber. By these two methods very nearly the same figures were obtained. Counts were made from the sediment in three tubes, which he designated R_1, R_2, R_3, corresponding to the maximal, mediate and minimal degrees of hemolysis; for example, one blood showed the following figures: In the sediment from the tube containing 0.5 per cent. NaCl solution were found 3 per cent. skeined forms, while from the tube containing 0.32 per cent. NaCl solution the sediment revealed 86.9 per cent. of skeined corpuscles. He obtained his highest increase by means of the pipet and counting chamber; in one blood the isotonic tube showed 0.37 per cent. of skeined forms, while a strongly hypotonic tube showed 71.9 per cent. In all these experiments the stain used was brilliant cresyl-blue, and this was added directly to the hemolytic fluid, thus probably increasing its hemolytic power a little. Ravenna concludes as the result of his findings that the corpuscle showing reticulation when vitally stained is the most resistant of all to hypotonic salt solutions, whereas the one showing granulations in fixed preparations is much less resistant, and this is explained, since the latter is a degenerative form, whereas the former is a young cell offering evidence of regeneration.

Luzzato and Ravenna,[19] a year later, used the same method in studying the autolysis of erythrocytes and found that the skeined forms are more resistant than many of the unskeined, but nevertheless when the skeined forms have all disappeared there are still a moderate number of unskeined forms not hemolyzed.

One of the difficulties in making accurate estimations in this work is the finding of large numbers of shadow corpuscles, even in the upper stages of hemolysis; and whereas these unskeined shadows are often overlooked, it is impossible to miss the skeined shadows with their bundle of dark-blue threads. This fact is probably very important and may readily account for some of the results reported by careless investigators, or by those who believe that such forms should be counted as unhemolyzed cells. Wladimir Alexieff[20] discussed this theoretically and described the

18. Ravenna, F.: Sulla resistenza delle emazie granulose nelle soluzioni chorosodiche. Lavori e riviste di chimica e microscopia clinica, 1909, i, 325.

19. Luzzatso, A. M., and Ravenna, F.: Sue fenomeni di autolisi nelle emazie granulose. Folia clinica, chemica et microscopica, 1910-11, iii, 53.

20. Alexieff, W.: Differences de structure et de signification des hématies granuleuses et des érythrocytes ponctués. Arch. d. mal. du coeur, etc., 1911, iv, 552.

phenomenon which he calls hemoglobinolysis, and which he distinguished from hemolysis. He believes hemoglobinolysis occurs more readily in skeined forms, and he advances in explanation that these forms have a liquid protoplasm which more readily gives up its hemoglobin. It is to hemoglobinolysis of these cells that he attributes the jaundice of congenital icterus and the prolonged stage of partial hemolysis seen in many hemolytic anemias. These hemoglobinolyzed forms thus persist with no further evidence of hemolysis and enter largely into the counts. As a result of an exhaustive study of the literature concerning reticulated corpuscles, only a small part of which is here briefly abstracted, one gets the general impression that the reticulated erythrocytes are more resistant to hemolytic agents than the unreticulated ones, and that this is explicable by their being young forms. It is, however, quickly discovered that few have made any careful attempts to investigate the matter and that except in Ravenna's work scarcely any figures are quoted, and only very general statements are made.

We have had occasion in connection with our study of reticulated erythrocytes to attempt to demonstrate an associated increased resistance, but we have not been able to do so with any degree of constancy. In all our experiments, rabbits were employed and we have studied their blood, both before and after various degrees of anemia had been produced by repeated injections of phenylhydrazine hydrochlorid. In the normal blood, the skeins were constantly present in less than 2 per cent. of the cells, and the sediment of tubes with varying degrees of hemolysis failed to show an increased percentage of these forms. In the anemic blood of the experimental animals, where the number of skeins was large (60 to 70 per cent.), we quite often obtained a moderate increase of percentage of reticulated forms after hemolysis, but with no constancy, and never were we able to demonstrate any such enormous changes as those reported by Ravenna. In these experiments we routinely used brilliant cresyl-blue as the vital stain, although at times experimenting with other basic stains, and we applied this stain according to both methods used by Ravenna and also according to several minor modifications, but without effect. In studying the hemolysis in the rabbits poisoned with phenylhydrazine it was found that erythrocytic resistance was often so increased that a very low percentage of salt solution had to be employed to produce marked hemolysis, but no marked increase of skeins in the sediment was ever seen. Counts made from each tube of a series varying by intervals of 0.05 per cent. NaCl from above the point of beginning hemolysis to complete hemolysis often showed only the slightest and inconstant variations. At the lower end of such a series the percentage of shadow forms containing skeins or of skeins with no remnant of corpuscles around them would often appear enormous, but it is certainly incorrect to speak of

such isolated skeins, or even of skeins in shadows as being cells more resistant than the others. The isolated skein is no more truly an evidence of resistance of the cell than the Heinz body. Without going into our work any further it can be stated that by using all the means at our disposal we were unable to demonstrate any increase of resistance of the reticulated erythrocytes to hypotonic salt solution. Nor does it seem very reasonable that there should be any such increase when one considers in what conditions an increase of skeined forms are seen. It is true that in secondary anemias both resistance and skeins are increased, but this is not always the case in pernicious anemia; while in congenital icterus, the condition in which the number of skeins is most marked, not only does the increase of resistance fail, but a true decrease of resistance is demonstrable. Similarly there are conditions in which we see an alteration in one or the other, that is, an alteration of the number of the skeins or an alteration in the fragility of the erythrocytes, without any change from the normal in the other characteristic of the cells. It seems probable, therefore, that the reticulated erythrocytes show no constant increase or decrease of fragility as compared with the unreticulated erythrocytes of the same blood.

Theoretically, it may be advanced that as the skeined forms are the younger cells they will in a more marked degree show, or will be the first to show, the alteration of resistance which the erythrocytes are undergoing in response to some stimulus; but whatever resistance they possess as reticulated forms will probably remain the same when they have become mature unreticulated forms.

SUMMARY

1. Of the intra-erythrocytic phenomena demonstrated by vital staining, that one known as reticulation of the erythrocytes is most important.

2. Reticulation of the erythrocyte is an evidence of youth of the cell.

3. A study of the literature of this subject leads to the belief that reticulated erythrocytes show greater resistance to hemolytic agents than do unreticulated forms. No consistent satisfactory proof of this has, however, been presented.

4. Our own experiments have failed to demonstrate any constant difference between the resistance of the reticulated and non-reticulated erythrocytes of rabbit's blood under normal and experimental conditions.

SIMPLIFIED METHODS FOR QUANTITATIVE ESTIMATION OF CHLORIDS IN THE URINE*

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The process generally followed to determine the amount of chlorids in the urine is precise. It is, nevertheless, complicated and time-consuming, even for the operator in the clinical laboratory. In addition, requiring the use of a buret, volumetric flask, volumetric pipets, filters and porcelain dishes, it demands a quantity of calibrated apparatus, which makes the method almost inaccessible to the general practitioner. The need of a simpler procedure, by which the usefulness of chlorid estimations might be made general, was recognized in Europe some years ago. Achard and Thomas,[1] in France, and H. Strauss,[2] in Germany, have introduced short methods comparable to the technic of albumin determinations by the Esbach tube, which in their opinion greatly facilitate chlorid estimations without sacrificing the degree of accuracy necessary for the diagnostic value of these analyses. Little use, however, seems to have been made of their work, and no references to the repetition or confirmation of their results are available. In order, therefore, to determine whether quantitative chlorid determinations can be made by methods which promise much in convenience, while losing little in precision, I have carried out, under the direction of Dr. Guthrie, the following studies of the methods and instruments of the authors mentioned above.

The two chief processes in use for the quantitative estimation of the chlorids in the urine are the Volhard[3] method and the method of Mohr.[4] On the former, the simplified method of Strauss is based; on the latter, the method of Achard and Thomas. The former of these will be described first, leaving the latter to be considered in the description of the work of Achard and Thomas.

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1. Achard, Ch., and Thomas, L.: Bull. et mém. Soc. méd. d. hôp. de Paris, 1912, xix, 596.
2. Strauss, H.: Praktische Winke fur die chlorarme Ernährung. Berlin, 1910.
3. Volhard: Jour. f. prakt. Chem., 1874, ix, 217.
4. Mohr: Lehrbuch der Titrirmethode., 1862, p. 317.

FILTRATION TO REMOVE SILVER CHLORID UNNECESSARY

The Volhard[3] method, as modified by Drechsel[5] and applied to the urine by Falk[6] and Arnold,[7] consists in the precipitation of the chlorids by an excess of a standardized solution of silver nitrate, the removal of the silver chlorid by filtration and the titration of the excess of silver nitrate with ammonium thiocyanate, using an iron salt as an indicator. This method, modified only as regards the strength of the solutions employed, later was applied to gastric and urinary analysis by Lüttke and Martius.[8] The Lüttke-Martius method is the one used in this laboratory and has been the one employed in these experiments. While this method is accurate, it demands a considerable amount of calibrated apparatus, and requires more than thirty minutes for duplicate determinations. The original Volhard method, however, could be carried out more rapidly than this, as it did not include the removal of the silver chlorid by filtration, the titration with ammonium thiocyanate being done in its presence. In using this method, Drechsel,[5] and more recently Rosanoff and Hill,[9] found difficulty with the end-point, due, they thought, to a reaction between the silver chlorid and the ammonium thiocyanate. To obviate this, Drechsel introduced the step of filtration, which has since been incorporated in this method and its modification by Lüttke and Martius. As the omission of the removal of the silver chlorid is one of the means employed by Strauss in simplifying this technic, it becomes important to determine whether this step has any influence on the results of the titration. Volhard[10] subsequently showed the error in Drechsel's criticism, and Goodall,[11] recently, in a study of sixty urines of widely varying chlorid content, has found that the original Volhard is as accurate as the Volhard modified by the filtration step. Harvey[12] has reached a similar conclusion from numerous experiments on the reaction between these solutions. He has found that in the Volhard method the precipitated silver chlorid does not react with the ammonium thiocyanate while an excess of silver nitrate is present; and further, that after the excess of silver has been removed, the reaction between the silver chlorid and the thiocyanate is markedly retarded by the presence, first, of the ferric salt, and secondly, by the excess of nitric acid in which the silver nitrate is dissolved. To verify this point, I made the determinations shown in Table 1.

5. Drechsel, E.: Jour. f. prakt. Chem., 1877, 191.
6. Falk, A.: Berichte d. deutsch. chem. Gesellsch., 1875, viii, 12.
7. Arnold, C.: Arch. f. d. ges. Physiol., 1885, xxxv, 541.
8. Lüttke and Martius, F.: Die Magensaüre des Menschen, Stuttgart, 1892. (Quoted by Strauss and others.)
9. Rosanoff and Hill: Jour. Am. Chem. Soc., 1907, xxix, 269.
10. Volhard: Liebig's Ann., 1877, exc, 1.
11. Goodall, H. W.: Boston Med. and Surg. Jour., 1909, clx, 304.
12. Harvey, S. C.: The Archives Int. Med., 1910, vi, 12.

TABLE 1.—Chlorid Determinations by Lüttke-Martius Method

Urine	AgCl Removed by Filtration		AgCl Present		Difference in Grams
	Gm. NaCl in 100 c.c.	Average	Gm. NaCl in 100 c.c.	Average	
I	0.67860	.....	0.67860	.....	.....
	0.67860	0.67860	0.678015	0.678307	0.00293
II	0.3888025	.....	0.390195	.....	0.0013925
III	0.8541	.....	0.857025	.....	0.002925

The results summarized in Table 1 show that filtration to remove the silver chlorid is not necessary under the conditions outlined above. As will be seen later, the factors in the analysis by the Strauss method are exactly similar to these.

LÜTTKE-MARTIUS METHOD

As the Volhard, and therefore the Lüttke-Martius, method has been proved accurate, the results obtained by the Lüttke-Martius procedure have been taken as the standard for gauging the accuracy of the other methods tested in these studies. This method, differing from its prototype only in the strength of the solution employed, is based on the precipitation of chlorids by silver nitrate, and the titration of the excess of silver nitrate by ammonium thiocyanate, using an iron salt as an indicator. Two standardized solutions are required:

1. N/10 $AgNO_3$ — an acid solution containing iron ammonium alum. One cubic centimeter of this standardized solution will precipitate 0.00585 gm. NaCl.
2. N/10 NH_4SCN — containing 7.6 gms. of ammonium thiocyanate to the liter of solution.

The preparation of these standard solutions will be described in detail in the consideration of the Strauss method.

To make a determination by this method, the procedure is as follows:

By means of a volumetric pipet, 10 c.c. of urine are placed in a 100 c.c. volumetric flask. To this are added 20 c.c. of N/10 $AgNO_3$ from a volumetric pipet. This mixture is shaken gently and allowed to stand for five minutes, until the precipitation of chlorids is complete. Distilled water is then poured into the flask, filling it to the 100 mark. The flask is stoppered, shaken well, and allowed to stand for a few minutes. If a reddish color appears in the mixture, as is nearly always the case with highly colored febrile urines, this is cleared by adding three or four drops of a solution of potassium permanganate. The mixture is filtered to remove the silver chlorid. Then, 50 c.c. of the filtrate are put into an evaporating dish by means of a volumetric pipet, and into this N/10 NH_4SCN is allowed to run, drop by drop, from a buret. At first, a brown color appears, due to the formation of ferricyanate. But in the presence of an excess of silver nitrate this immediately disappears, giving place to a bluish white precipitate of silver cyanate. The titration is continued until a permanent reddish brown color appears in the fluid. This color, due to the formation of

ferricyanate, occurs permanently when all of the silver has been precipitated. The reading of the buret at this point denotes the excess of silver in 50 c.c., or half of the amount of fluid in the flask. This figure, therefore, must be multiplied by two in order to obtain the amount of the silver in excess in the total volume of the mixture. From this the chlorid content is calculated as follows: For example:

Buret reading shows 6 c.c. N/10 NH_4SCN used.

Therefore $6 \times 2 = 12 =$ excess of silver in total mixture. Then 20 c.c. N/10 $AgNO_3$ less $12 = 8 =$ c.c. of silver solution required to precipitate all chlorids in 10 c.c. of urine, and $8 \times 0.00585 = 0.0468 =$ gm. of NaCl in 10 c.c. urine. If the total output of the urine examined were 1 liter, then the total chlorids would be 4.68 gm. per 1,000 c.c.

The amount of silver here employed is capable of indicating at most 11.7 grams of chlorids per liter. Urine, found by a preliminary test to contain more chlorids than this, must be diluted, or a larger quantity of silver nitrate may be added, before the analysis can be made.

Fig. 1.—Strauss chloridometer.

THE STRAUSS METHOD

The chief simplification of this process has been introduced by Prof. H. Strauss[2] of Berlin. He has condensed into a single tube[13] all of the necessary calibrated apparatus, and claims simplicity together with sufficient clinical accuracy for his method of chlorid determinations. The author has not formally published the results which he has obtained by the use of this instrument, but in a letter has written me that he has been using it for some time with satisfaction. The method is simply the Lüttke-Martius process done in one piece of apparatus. The filtration

13. The Strauss Chloridometer is manufactured by Paul Altmann of Berlin. Price 2.50 marks.

to remove the precipitate of silver chlorid—a step already shown to be unnecessary—is omitted.

The Strauss instrument, shown in Figure 1, is a glass tube 20 cm. long and 2 cm. in diameter. Its lower end is broadened into a base, too small, however, to steady effectually the long cylinder. The upper end is fitted with a ground-glass stopper. Its barrel is calibrated with the marks shown in the figure, to indicate the volumes of silver nitrate solution and of urine to be placed in the tube. Above the mark for the urine, the tube bears a scale reading in 0.5 gm. from 11 gm. to 0.5 gm. These scale marks indicate grams per liter. Urine containing more than 11 gm. of chlorids per liter must be diluted before being analyzed by this method.

For the purpose of the analysis, two standardized solutions[14] are required:

1. N/10 $AgNO_3$ (Lüttke-Martius' silver solution).

This solution contains 16.966 gm. of silver nitrate to the liter. 1 c.c. of this solution will precipitate 0.00585 gm. NaCl. It is prepared as follows:

17.5 gm. $AgNO_3$,
900 c.c. HNO_3 (25 per cent.)
50 c.c. iron ammonium alum, a cold saturated solution, which is about a 10 per cent solution.
Distilled H_2O to 1,000 c.c.

An excess of silver nitrate is used because the salt is somewhat hygroscopic and hence the true amount of silver nitrate is not indicated by the weight of the substance. This solution is standardized in the usual way by titration against known normal hydrochloric acid, the iron salt in the solution acting as an indicator. Thus, 20 c.c. of the silver nitrate solution are added to 20 c.c. of known tenth normal hydrochloric acid. A solution of ammonium thiocyanate is added to this, and the silver solution adjusted until the end-point, or brownish red color, is given on the addition of a very small drop of the thiocyanate. The solution, as made up, will have to be diluted in the process of standardization. This dilution is done by adding a special fluid containing seven parts of distilled water, 2.5 parts of concentrated nitric acid, and 0.5 parts of a 10 per cent. solution of iron ammonium alum. In this way, as has been shown, the factors necessary for a sharp end-point are provided. When kept in a brown bottle, away from the light, this solution remains constant in strength for a long time.

2. N/20 NH_4SCN (3.8 gm. NH_4SCN per liter).

This solution is one-half the strength of the ammonium thiocyanate of the Lüttke-Martius method. Its greater dilution allows a scale of longer intervals to be marked on the tube. It is prepared by dissolving 4 gm. NH_4SCN in 1,000 c.c. of distilled water. Then, by titration with the standardized solution of silver nitrate, in which the iron salt acts as an indicator, it is adjusted by dilution, with water or the addition of more of the thiocyanate, as indicated, so that

2 c.c. N/20 NH_4SCN = 1 c.c. N/10 $AgNO_3$.

In order to make a chlorid estimation with the Strauss instrument, N/10 $AgNO_3$ (Lüttke-Martius' silver solution) is placed in the tube until the bottom of the meniscus corresponds to the mark A. Urine is added until the mark U is reached. These fluids are mixed by gently inverting the tube several

14. Prof. Strauss writes that he has been using such solutions as these prepared by Kahlbaum, and he has found them satisfactory. They are prepared also by pharmaceutical houses in this country.

times. Shaking, which causes the formation of bubbles, must be avoided. The tube is then allowed to stand for five minutes. If a reddish color develop in the mixture, as occurs with all but pale urines, 3 drops of a 10 per cent. solution of potassium permanganate are added. This decolorizes the fluid and the amount of potassium permanganate does not markedly raise the level of the fluid in the tube. In any case, allowance for the increase in the fluid may be made when the scale is read at the end of the titration. After five minutes, N/20 NH_4SCN is run gradually into the tube by means of a buret, pipet, or through a siphon from a bottle of the solution on a shelf. After each addition of the ammonium thiocyanate, the tube is inverted several times gently. As in the case of the Lüttke-Martius' titration, the reddish brown color which appears at first, immediately disappears as long as an excess of silver is present. The addition of ammonium thiocyanate is continued until a definite, permanent reddish-brown color in the fluid is obtained. This end-point, with solutions made up as prescribed, is sharp and lasting. It may be appreciated most readily by holding the tube against a white background. It is well to obtain a good reddish-brown color, for a drop or so of the thiocyanate makes little difference in the scale readings, but a great deal of difference in the intensity of the color. When the titration is complete, the amount of the chlorids in *grams per liter* is read off from the scale.

Experiments to test the accuracy of these instruments, and of the method, were carried out along the following lines:

(a) tests of the calibrations of the tube,
(b) estimations of chlorids in aqueous solutions,
(c) estimations of the chlorids in normal urine,
(d) estimation of the chlorids in urines containing albumin.

In all titrations, results obtained by the Lüttke-Martius method, were taken as the absolute for comparison.

(a) *Calibration of Strauss Tubes.*—The volumes contained between the various marks on Strauss tubes should be as follows: From bottom of tube to the mark A, 10 c.c.; from A to U, 5 c.c.; from U to 11, 1.2 c.c.; and between every mark thereafter on the scale, 0.9 c.c. The calibration of the six tubes imported by this laboratory was tested by comparison with the readings of a standardized buret. By this test, none of the tubes was found to be perfectly accurate. In some the errors were greater than in others, a discrepancy of as much as 0.2 c.c. between the tube-volumes and the absolute scale being noted. As a rule, however, the A and U volumes were found to be in the proper relation. The tubes are put out uncorrected and unaccompanied by any guarantee of accuracy. These errors of workmanship, which, on the whole, are not very large, are therefore to be expected. They do not detract from the value of the tube as a clinical instrument.

(b) *Estimation of Chlorids in Aqueous Solutions.*—Solutions containing sodium chlorid in amounts corresponding to all of the gradations on the apparatus were analyzed. The results of these determinations with the six Strauss tubes are presented in Table 2.

TABLE 2.—Estimation of Chlorids in Aqueous Solution by Strauss' Method Compared with Lüttke-Martius' Method

Grams NaCl per liter by						
Lüttke-Martius'	Strauss					
Method	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
0.5	0.5	0.3	0.3	0.5	0.3	0.6
1.228	1.3	1.5	1.5	1.5	1.3	1.4
1.638	1.8	2.05	2.	2.	1.8	2.
2.106	2.1	2.1	2.5	2.1	2.1	1.9
2.574	2.6	2.9	2.7	.....	2.7	3.
3.276	3.5	3.4	3.5	3.5	3.5	3.2
3.627	3.9	3.7	3.7	3.6	3.55	3.6
3.9953	4.	4.05	4.05	4.05	4.	4.4
4.797	4.75	4.75	5.	4.75	5.	4.75
5.0885	4.9	5.1	5.05	5.02	5.1	5.
5.5867	5.6	5.7	5.8	5.8	5.7	5.9
6.101	6.05	6.1	6.	6.1	6.	6.
6.5227	6.75	6.5	6.6	6.6	6.6	6.6
7.254	7.1	7.5	7.5	7.5	7.2	7.1
7.605	7.55	7.6	7.55	7.55	7.8	7.8
8.1607	8.1	8.1	8.15	7.8	8.2	8.
8.5702	8.6	8.7	8.7	8.6	8.5	8.8
9.0967	9.4	9.5	9.3	9.1	9.4	9.5
9.5355	9.55	9.8	9.55	9.5	9.5	10.
10.2667	10.	10.2	10.5	10.6	10.2	10.5
10.764	11.	10.7	11.	10.8	10.7	10.9
11.0565	11.1	11.1	11.2	11.4	11.05	11.3
Max. difference from Lüttke-Martius	0.3033	0.403	0.394	0.3435	0.3033	0.426
Min. difference	0.	0.001	0.0145	0.	0.0047	0.0115
Average difference	0.1516	0.202	0.2042	0.1717	0.154	0.2187

It is shown by this table that the results obtained by the Strauss method are somewhat at variance with the Lüttke-Martius determinations and also exhibit discrepancies between the different tubes. The greatest variation from the absolute (Lüttke-Martius) standard for any amount of chlorids was 0.426 gm. per liter; the smallest was 0. The average of these variations was approximately 0.2 gm. per liter. In nearly all cases the tubes indicated amounts too high. These discrepancies must be attributed to the errors in the calibration of the tubes and to the experimental error in reading the buret. Readings of approximately equal accuracy were obtained from all regions of the scale.

(c) *Estimation of Chlorid in Normal Urine.*—Normal urines of varying chlorid content were analyzed by the Lüttke-Martius and Strauss methods. The results of these tests are summarized in Table 3.

The results of these titrations of the chlorids in urine, even more than those of chlorids in aqueous solution, exhibit the satisfactory accuracy of the Strauss method. In this series (Table 3), the largest discrepancy between the Strauss estimation and the Lüttke-Martius was 0.349 gm. per liter, and this occurred in analyzing a urine containing more than 11 gm. of chlorids per liter. This reading was an approximate one, as the fluid in the tube was below the last scale mark. Even with this, however, the average mean of error was 0.1343 gm. per liter. While this error would be very large in a purely chemical analysis, it is negligible in a clinical estimation. On these results, therefore, it may be concluded that the Strauss method applied to the normal urine is amply accurate to serve as a basis for clinical diagnosis.

TABLE 3.—Strauss Tube Estimation of Chlorids of Normal Urine

Method	Grams NaCl per Liter					
Lüttke-Martius'	2.0475	4.9725	6.903	7.722	9.716	11.349
Strauss—						
Tube 1	2.05	4.8	6.9	7.8	9.5	11.5
Tube 2	2	4.7	7	7.6	9.8	11.5
Tube 3	2	.....	6.6	7.7	9.8	11.1
Tube 4	1.9	.....	.....	7.6	9.6	11
Tube 5	.....	5	7.05	7.6	9.7	11.5
Tube 6	.....	5	.....	7.7	9.8	11.5
Max. difference	0.1475	0.2725	0.303	0.122	0.216	0.349
Min. difference	0.0035	0.0275	0.003	0.022	0.016	0.151
Mean difference	0.0755	0.15	0.153	0.072	0.1165	0.250

Several of the urines in the above series were so highly pigmented that their color interfered with the sharp appearance of the end-point. They were, however, readily decolorized by adding three or four drops of a 10 per cent. solution of potassium permanganate to the mixture in the tube a few minutes before titration. As a rule, the color which develops while the urine stands in contact with the strongly acid silver nitrate solution is made sufficiently pale by dilution from the subsequent addition of the ammonium thiocyanate. But even in normal urine this color may obscure the end-point. It is well, therefore, whenever working with a urine of a deep yellow color to decolorize the mixture in the tube by adding a few drops of the solution of potassium permanganate. Whatever increase in the level of the fluid in the tube is caused by this may be discounted in reading the scale. The advantage of a much more distinct end-point is gained by this step.

(d) *Estimation of Chlorids in Albuminous Urines.* — Although Arnold,[7] in estimating the chlorids in a variety of fluids — milk, blood-serum and urines containing albumin — showed that the presence of

protein in the fluid under analysis did not interfere with the accuracy of the Volhard method, it was considered well to repeat some of his work and to determine whether the Strauss procedure would be disturbed by the presence of protein. It is conceivable that some of the silver of the N/10 silver nitrate solution might become bound to the albumin, forming a silver albuminate, and thus be unavailable in the titration. To test this point solutions of chlorid-free protein of varying concentration were added to normal urine of known chlorid-content and of constant volume, and the resulting mixture was titrated as in the foregoing experiments. The salt-free protein was obtained from blood-serum by dialyzing the serum against changes of distilled water until the water around the dialyzing sac no longer gave a cloudiness with silver nitrate, and the protein in the sac began to precipitate. In this way, solutions of protein were prepared containing from 0.7 to 30 gm. of protein per liter (Tsuchiya). These solutions were shown to be salt-free by Lüttke-Martius determinations. When 10 c.c. of the protein solution were added to 20 c.c. N/10 $AgNO_3$, and the mixture titrated for the excess of silver, the result was found to correspond exactly with what was obtained when those amounts of the standard solutions were titrated. The solutions balanced.

In these tests, however, a heavy yellowish precipitate was formed when the protein was added to the strongly acid silver solution. This precipitate interfered with the analysis only by its bulkiness, when more than 30 gm. of protein per liter were present, but it did not at all disturb the balanced reaction between the standardized silver nitrate and ammonium thiocyanate. The results of a series of titrations with urines containing proteins are given in Table 4.

From Table 4 it is evident that in the time in which the analysis is performed, none of the silver of the standardized solution becomes bound to the protein in such a manner as to render it unavailable for the titration. This table shows that the greatest discrepancy between the Lüttke-Martius and Strauss results was 0.343 gm. of chlorids per liter, and that this occurred only in the analysis of the urine containing 27 gm. of protein per 1,000 c.c. An error as large as this was to be expected from the previous experiments with protein-free urine. But as regards the precipitation of the silver in combination with the protein, the most significant fact is that chloridometry by the Lüttke-Martius method on equal quantities of the same urine, free from or containing protein, gives exactly equal results. Thus by these analyses of urine rendered artificially pathological under controllable conditions, it is shown that the presence of protein in amounts up to 27 gm. per liter does not interfere with the accuracy of the Strauss method.

TABLE 4.—STRAUSS TUBES WITH ALBUMINOUS URINES

URINE A

Tests	25 c.c. Normal Urine. 50 c.c. Water	25 c.c. Same Urine 50 c.c. Protein A	Same Mixture Protein Removed. Vol. Not Restored
Heat and acetic ...	0	+	0
Tsuchiya	*	‡0.7	*
Lüttke-Martius ...	†4.446	4.446	4.5045
Strauss —			
Tube 1	4.2	4.2	4.5
Tube 2	4.5	4.3	4.5

URINE B

Tests	50 c.c. Urine. 50 c.c. H_2O	50 c.c. Same Urine 50 c.c. Protein B	Same Mixture Protein Removed. Vol. Not Restored
Heat and acetic ...	0	+	0
Tsuchiya	*	1.4	.....
Lüttke-Martius ...	5.7330	5.7330	5.9670
Strauss —			
Tube 1	5.6	5.7	6
Tube 2	5.7	5.6	6.05

URINE C

Tests	25 c.c. Urine. 50 c.c. H_2O	25 c.c. Urine 50 c.c. Protein C	Same Mixture Protein Removed. Vol. Not Restored
Heat and acetic ...	0	+	0
Tsuchiya	0	10	0
Lüttke-Martius ...	5.2065	5.265	5.3235
Strauss —			
Tube 1	5.1	5.05	.....
Tube 2	5.2	5.2	.....

URINE D

Tests	10 c.c. Urine 90 c.c. H_2O	10 c.c. Urine 90 c.c. Protein D	Same Mixture Protein Removed. Vol. Restored
Heat and acetic ...	0	+	0
Tsuchiya	.....	27	0
Lüttke-Martius ...	2.6910	2.6432	2.2113
Strauss —			
Tube 1	2.8	2.3	2.3
Tube 2	2.6	2.5	2.3

* Not done.
† Chlorids expressed in grams per liter.
‡ Protein expressed in grams per liter.

A source of large error was introduced when the protein was removed from the urine by heat, acetic acid and filtration. The results of titration made after this manipulation are shown in the third column of Table 4. When the estimations are made on the filtrate not restored to the original volume, the amount of chlorids found is too high. But when the analyses are made on the filtrate restored to its original volume by the addition of water, the amounts are too low. The loss of water by evaporation, the carrying down or mechanical "locking up" of chlorids by the heavy precipitate of protein cannot be compensated for by dilution. It is better, therefore, to make the titrations directly in the presence of the protein contained in albuminous urine.

On the basis of the numerous tests described above it may be concluded that the Strauss method is an improvement on the usual methods for determining chlorids. From a clinical standpoint it is a simple, rapid and sufficiently accurate method for determining chlorids in both normal and pathological urine.

CHLORIDOMETRY IN GRADUATED CYLINDERS BY STRAUSS METHOD

Although the Strauss method is a simple and commendable one, it nevertheless retains some points of inaccessibility, as the instrument employed is of foreign manufacture, requiring importation and demanding a rather high price. It seemed well, therefore, to attempt a further simplification of the process. The standardized solutions, obviously, cannot be eliminated. Two of these are required in the Strauss method, but when a laboratory is able to prepare one standardized solution another can be made with only little additional difficulty. The instrument, however, can be simplified. In place of it, a simple graduated 50 c.c. cylinder may be used, and with such a "tube" and a scale for interpreting its results, sufficiently accurate chlorid estimations may be made, as is shown in Table 5.

In making determinations the same solutions and the same amounts of them were put into the cylinders as were used in the Strauss tubes. From the amounts of the various fluids employed, it is evident that when an end-point is reached in the titration, a definite volume of fluid is present in the tube. Thus, for example, it was seen by actual measurement that when the end-point was reached with 5 gm. per liter the Strauss tube contained 26.45 c.c. of total fluids. It follows, moreover, from the volume of silver nitrate solution, the urine and the ammonium thiocyanate, and from their numerical relationships, that the total volume of fluid present for any degree on the scale of chlorids may be calculated. In this way, total volume of fluid may be converted into grams of chlorids per liter, or *vice versa*. For this purpose the following formula has been derived:

Grams chlorids per liter = 20.475 — 0.585 V. Eq. I where V is the total fluid present in the tube.

The formula is derived as follows:

Solutions placed in tube:

5 c.c. or 1/200 of a liter of urine

10 c.c. N/10 $AgNO_3$. Each c.c. of N/10 $AgNO_3$ will precipitate 0.00585 gm. NaCl.

Sum of these solutions = 15 c.c.

N/20 NH_4SCN, of which 2 c.c. N/20 NH_4SCN = 1 c.c. N/10 $AgNO_3$.

V = total fluid at end of titration.

V — 15 = amount of N/20 NH_4SCN used in titration.

V — 15 = Excess of N/10 $AgNo_3$ present after precipitation of chlorids.

$$10 - \frac{V - 15}{2} = \text{amount of N/10 } AgNO_3 \text{ used in precipitation of chlorids.}$$

Then

$$\left[10 - \frac{V - 15}{2}\right] 0.00585 = \text{gm. NaCl in 5 c.c. urine.}$$

$$\left[10 - \frac{V - 15}{2}\right] 0.00585 \times 200 = \text{gm. NaCl in 1,000 c.c. urine.}$$

When simplified, this expression is

20.475 — 0.585 V = gm. NaCl per liter of urine. Eq. I.

Example:

Suppose total fluid at end of titration = 21.35 c.c.

Substituting in Equation I:

20.475 — 0.585 x 21.35 = gm. chlorids per liter.

$\therefore$ 7.98525 = gm. chlorids per liter.

In this case the Lüttke-Martius determination showed that the chlorids were 7.956 gm. per liter.

By a rearrangement of the terms of this formula, an expression is derived by which the amount of fluid present may be calculated from the number of grams of chlorids per liter in the solution analyzed. This formula would be useful in constructing or checking the calibration of an instrument:

$$V = \frac{20.475 \text{ gm. chlorid per liter}}{0.585}$$ Equation II, where V is the total fluid in the cylinder at the end of the titration.

From Equation I a scale has been computed for use with the graduated cylinders, from which the numbers of grams of chlorids per liter of the urine, or solution tested, may be read off from the total amount of fluid present at the end of the titration. This scale has been calculated in 0.1 gm. NaCl per liter from 0 to 11.7 gm. per liter. Whereas the scale on the Strauss tube registers from 11 to 0.5 gm. per liter, this scale somewhat extends these limits. By means of it, a range of 1.2 gm. per liter beyond the limits of the Strauss scale is allowed for chlorid estima-

tions in the graduated cylinders. The calculated values of the scale correspond quite accurately to the amounts observed when testing volumetrically the calibrations of the Strauss tubes.

To test this method, a series of determinations were made with ordinary 50 c.c. cylinders chosen at random from the supply in the laboratory and with a certified cylinder of an accurate calibration. The graduations on the ordinary cylinders were for 1 c.c., extending over a tube length of 12.5 cm. The certified cylinder was graduated in 0.2 c.c., extending over a scale length of 25 cm. For these experiments the

SCALE OF CHLORIDOMETRY IN CYLINDERS

Chlorids Gm. per Liter	Volume	Gm.	Volume	Gm.	Volume	Gm.	Volume	Gm.	Volume	Gm.	Volume
0.0	35.000	2.0	31.58	4.0	28.16	6.0	24.74	8.0	21.32	10.0	17.90
0.1	34.829	2.1	31.409	4.1	27.999	6.1	24.569	8.1	21.159	10.1	17.739
0.2	34.671	2.2	31.238	4.2	27.828	6.2	24.398	8.2	20.988	10.2	17.568
0.3	34.487	2.3	31.060	4.3	27.657	6.3	24.227	8.3	20.717	10.3	17.397
0.4	34.316	2.4	30.896	4.4	27.486	6.4	24.056	8.4	20.546	10.4	17.126
0.5	34.128	2.5	30.72	4.5	27.30	6.5	23.88	8.5	20.48	10.5	17.04
0.6	33.971	2.6	30.549	4.6	27.129	6.6	23.709	8.6	20.309	10.6	16.869
0.7	33.803	2.7	30.378	4.7	26.958	6.7	23.538	8.7	20.138	10.7	16.698
0.8	33.632	2.8	30.207	4.8	26.787	6.8	23.367	8.8	19.967	10.8	16.527
0.9	33.461	2.9	30.036	4.9	26.616	6.9	23.196	8.9	19.796	10.9	16.356
1.0	33.29	3.0	29.87	5.0	26.45	7.0	23.03	9.0	19.625	11.0	16.19
1.1	33.119	3.1	29.699	5.1	26.279	7.1	22.869	9.1	19.454	11.1	15.919
1.2	32.948	3.2	29.528	5.2	26.108	7.2	22.698	9.2	19.283	11.2	15.748
1.3	32.771	3.3	29.357	5.3	25.937	7.3	22.527	9.3	19.112	11.3	15.577
1.4	32.606	3.4	29.186	5.4	25.766	7.4	22.356	9.4	18.941	11.4	15.406
1.5	32.435	3.5	29.025	5.5	25.605	7.5	22.175	9.5	18.755	11.5	15.33'
1.6	32.264	3.6	28.854	5.6	25.434	7.6	22.004	9.6	18.584	11.6	15.171
1.7	32.093	3.7	28.683	5.7	25.263	7.7	21.833	9.7	18.413	11.7	15.000
1.8	31.912	3.8	28.512	5.8	25.092	7.8	21.662	9.8	18.242	....	.....
1.9	31.741	3.9	28.341	5.9	24.921	7.9	21.491	9.9	18.071	....	.....

cylinders were closed with rubber stoppers. Titrations were made in these cylinders according to the Strauss method and the results compared with Lüttke-Martius and Strauss determinations on the same urines. The results of these analyses are shown in Table 5. In this table also are given the results of this method obtained when smaller cylinders (10 c.c.) were used. The significance of this will be described later.

Considering for the present only the results of titration in the 50 c.c. cylinders, it is seen from Table 5 that errors in chloridometry with the ordinary cylinders range from 0.149 to 0.271 gm. of chlorids per liter, while errors with the standardized cylinder are less, being from 0.021 to 0.12 gm. per liter of urine. The larger errors of the ordinary cylinders are explained by the errors found to be existent in their calibrations

when the supposed cubic content between the graduations was measured by means of a buret. The more accurate results with the certified cylinder were to be expected from the accuracy of its calibration and the

TABLE 5.—ESTIMATION OF CHLORIDS IN URINE BY STRAUSS METHOD IN GRADUATED CYLINDERS. RESULTS COMPARED TO STRAUSS TUBE AND LÜTTKE-MARTIUS DETERMINATIONS

Method	Tube and Lüttke-Martius Determinations				In Gm. per liter, Difference from Lüttke-Martius
	Gm. per liter Observed	Gm. per liter by Scale	Volume c.c. Observed	Volume c.c. by Scale	
Lüttke-Martius	0.2340	.....	.....	34.6	.....
Strauss tube	Meniscus above scale	.....	.....	.....	.....
Ordinary 50 c.c. cyl.	.....	0.5	34.1	.....	+0.266
Certified 50 c.c. cyl.	.....	0.15	34.75	.....	—0.084
Ordinary 10 c.c. cyl.	.....	0.75	6.75	.....	+0.516
Certified 10 c.c. cyl.	.....	0.25	6.85	.....	+0.016
Lüttke-Martius	0.7371	.....	.....	33.73	.....
Strauss tube 2	0.7	.....	33.6	.....	—0.0371
50 c.c. cyl. ord.	.....	0.45	34.2	.....	—0.1871
50 c.c. cyl. cert.	.....	0.8	33.6	.....	+0.0629
10 c.c. cyl. ord.	.....	1.45	6.5	.....	+0.7189
10 c.c. cyl. cert.	.....	0.75	6.75	.....	+0.0189
Lüttke-Martius	4.329	.....	.....	27.6	.....
Strauss tube 4	4.2	.....	27.88	.....	—0.129
50 c.c. cyl. ord.	.....	4.6	27.1	.....	+0.271
50 c.c. cyl. cert.	.....	4.35	27.5	.....	+0.021
10 c.c. cyl. ord.	.....	5.1	5.25	.....	+0.771
10 c.c. cyl. cert.	.....	4.25	5.55	.....	—0.079
Lüttke-Martius	8.5702	.....	.....	20.35	.....
Strauss tube 1	8.6	.....	20.45	.....	+0.0298
50 c.c. cyl. ord.	.....	8.4	20.5	.....	—0.1702
50 c.c. cyl. cert.	.....	8.6	20.3	.....	+0.0298
50 c.c. cyl. ord.	.....	9.05	3.9	.....	+0.5098
10 c.c. cyl. cert.	.....	8.7	4.00	.....	+0.1298
Lüttke-Martius	10.530	.....	.....	17.00	.....
Strauss tube 2	10.6	.....	16.89	.....	+0.07
50 c.c. cyl. ord.	.....	10.7	16.6	.....	+0.17
50 c.c. cyl. cert.	.....	10.65	16.7	.....	+0.12
10 c.c. cyl. ord.	.....	11.05	3.2	.....	+0.52
10 c.c. cyl. cert.	.....	10.8	3.3	.....	+0.27
Lüttke-Martius	11.349	.....	.....	15.6	.....
Strauss tube 2	Meniscus below scale	.....	.....	.....	.....
50 c.c. cyl. ord.	.....	11.1	15.9	.....	—0.149
50 c.c. cyl. cert.	.....	11.25	15.7	.....	—0.099
10 c.c. cyl. ord.	.....	11.3	3.1	.....	—0.049
10 c.c. cyl. cert.	.....	11.4	3.15	.....	+0.051

length and numerous graduations of its scale. With both kinds of cylinders, chlorid determinations can be made with accuracy equal to that of the Strauss tubes, and the advantages of their longer scales are shown

by the first and last analyses of the series in Table 5, in which the meniscus was once above and once below the limits of the Strauss scale. For chlorid estimations by this rapid method, the certified 50 c.c. cylinder is to be recommended especially. It has the advantage of being easily procured, of having accurate calibrations and a long, frequently divided scale, and in addition gives results that are considerably more accurate than those obtained by the Strauss tubes.

As the estimation of chlorids in the urine has diagnostic value in the study of renal function, as well as in the study of many diseases, an attempt was made to adapt the method of chloridometry in graduated cylinders to the requirements of urology. In studying renal function by means of collecting through a ureteral catheter the urine from one kidney, often only 1 c.c., or slightly more than this, is obtained. For the ordinary Strauss test this amount of urine is insufficient, and it is likewise not enough for the test when carried out in 50 c.c. cylinders. For this reason small 10 c.c. cylinders were employed in the series of analyses summarized in Table 5. One cylinder was an ordinary 10 c.c. graduated cylinder, the calibrations of which were inaccurate. The other was a somewhat longer 10 c.c. cylinder, graduated in 0.1 c.c., with accurate calibration. Into these cylinders 1 c.c. of urine and 2 c.c. N/10 $AgNO_3$ (Lüttke-Martius silver solution) were placed. The titration was then done by adding N/20 ammonium thiocyanate. In this way the relations between the solutions were kept the same as in the ordinary Strauss method. As one-fifth of all the solutions used in computing the scale for chloridometry in graduated cylinders, the results obtained with the 10 c.c. cylinders had only to be multiplied by 5 in order to allow reading from the amount of fluids in the cylinders the grams of chlorids per liter of urine tested, by means of the scale computed for the larger cylinders. Determinations made by these small cylinders, however, exhibited large discrepancies from the Lüttke-Martius figures. With the ordinary 10 c.c. cylinders errors ranged from 0.049 to 0.7189 gm. of chlorids per liter, while with the certified 10 c.c. cylinders they were from 0.016 to 0.27 gm. per liter. With the certified cylinder the readings were within the limits of desired clinical accuracy. But, in spite of this, these small cylinders cannot be recommended for use in making chlorid estimations. Great care must be exercised in putting the urine and silver nitrate solution in the cylinders, for when such small quantities are used, a drop of the solution left along the sides of the tube will have large effect on the result of the titration. It is advisable, therefore, when only small amounts of urine to be analyzed for chlorids are available, to dilute this urine carefully a sufficient number of times to give somewhat more than 5 c.c. More accurate estimations then can be made on the diluted urine by means of the Strauss method in the tubes or in the graduated cylinders than are possible with small cylinders, using small quantities of urine.

THE METHOD OF ACHARD AND THOMAS

The other simple method for the determination of chlorids in the urine is that of Achard and Thomas.[1] These authors have devised an instrument comparable to the Esbach tube, and employ the process of Mohr[4] for the titration method. This method is based on the precipitation of chlorids by silver nitrate of known strength added in excess, and the titration for the excess of silver using potassium chromate as an indicator. In Mohr's procedure a certain amount of the urine is placed in a dish, to which is added, drop by drop, a standardized solution of silver nitrate (29.075 gm. $AgNO_3$ dissolved in 1,000 c.c. H_2O; of this solution, 1 c.c. will precipitate 0.01 gm. NaCl). To denote when all the chlorids of the urine have been precipitated a drop or two of a solution

Fig. 2.—Chloridometer of Achard and Thomas.

of potassium chromate are added as an indicator. When the precipitation of chlorids is complete, a reaction takes place between the silver and the chromate, forming silver chromate, which gives a brownish-red color to the mixture. The appearance of the color indicates the end of the reaction, and at this point the amount of chlorids present in the urine is determined from the quantity of the silver used.

The approximate estimation of the chlorids which Achard and Thomas have introduced, rests on the same reaction, but proceeds in the opposite direction. Instead of adding to a fixed amount of urine a variable quantity of silver nitrate, the quantity of urine is varied, while that of the silver nitrate remains constant. This conveniently permits employing only a small amount of the standardized solution of silver

nitrate, and on this basis the method is recommended by the authors as a bedside procedure. The potassium chromate is added to the silver nitrate in the tube before the addition of any urine. Silver chromate is formed at once, giving the mixture a deep red color. To this, the urine is added and the tube inverted several times. The chromate of silver is decomposed by the successive addition of urine and silver chlorid and a yellowish precipitate is formed. The end of the reaction occurs when all of the reddish-brown color disappears, and gives place to a light yellow color throughout the fluid and precipitated contents of the tube. For this test the urine should be slightly acid, rendered so, if necessary, by the addition of a little acetic acid.

The instrument[15] shown in Figure 2, employed by Achard and Thomas, is a tube not unlike the Strauss chloridometer. This tube is closed by a rubber stopper; its barrel bears a special calibration to indicate the amount of chlorids in the urine thus tested. At the bottom, a first graduation, marked A, indicates the fixed quantity of the standard solution of silver nitrate to be placed in the tube. The quantity is 5 c.c. Above this mark, the graduations of the scale indicate in grams per liter various amounts of chlorids.

The scale is constructed according to the following table:

3.3	c.c.	correspond to	15.0	gm. chlorids per 1,000 c.c. of urine.
5.0	c.c.	correspond to	10.0	gm. chlorids per 1,000 c.c. of urine.
7.1	c.c.	correspond to	7.0	gm. chlorids per 1,000 c.c. of urine.
10.0	c.c.	correspond to	5.0	gm. chlorids per 1,000 c.c. of urine.
12.5	c.c.	correspond to	4.0	gm. chlorids per 1,000 c.c. of urine.
14.0	c.c.	correspond to	3.5	gm. chlorids per 1,000 c.c of urine.
16.0	c.c.	correspond to	3.0	gm. chlorids per 1,000 c.c. of urine.
20.0	c.c.	correspond to	2.5	gm. chlorids per 1,000 c.c. of urine.

The total volume in the tube at any of these degrees is the amount given here plus the 5 c.c. of silver nitrate solution.

To make determinations with this tube the following procedures must be carried out:

The tube is filled to the mark A with the standardized solution of silver nitrate (29.075 gm. $AgNO_3$ to 1,000 c.c. H_2O). To this 3 or 4 drops of a 1:5 solution of potassium chromate are added. The undiluted urine is then added gradually, mixing the fluids constantly by gentle inversion of the tube. When the reddish-brown color of the mixture finally changes to a permanent yellow, the end-point is reached. The chlorids present in grams per liter are read off directly from the scale.

The authors advise using the upper limit of the scale because of the greater accuracy of readings in these regions. If the amount of chlorids in the urine is above 5 gm. per liter on the first test, a second determination should be made with diluted urine. Above 5 gm. of chlorid per

15. The Achard and Thomas chloridometer is manufactured by Paul Altmann of Berlin. Prince 3 marks.

liter make dilutions of 1 to 2, above 10, a dilution of 1 to 4, above 15, dilute 1 to 6. The results thus obtained are to be multiplied by the amount of the dilution to estimate the quantity of chlorids in the undiluted urine. When the chlorids are less than 2.5 gm. per liter, the silver nitrate must be diluted; when, to calculate the true chlorid content of the urine, the observed content should be divided by the degree of the dilution.

TABLE 6.—ESTIMATION OF CHLORIDS IN AQUEOUS SOLUTIONS BY ACHARD-THOMAS METHOD COMPARED WITH LÜTTKE-MARTIUS METHOD

Grams NaCl per liter by						
Lüttke-Martius Method	Achard-Thomas					
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
0.5	0.483	0.5	0.583	.....	.....	.....
1.228	1.03	1.0	1.0	1.0	1.0	1.03
1.638	1.33	1.25	1.666	1.5	1.33	1.33
2.106	1.91	1.91	1.8	2.16	2.	1.91
2.574	2.7	2.8	2.75	2.8	2.75	2.8
3.276	3.2	3.25	3.2	3.25	3.2	3.2
3.627	3.6	3.5	3.7	3.55	3.55	3.55
3.9953	4.1	4.05	4.05	4.1	4.1	4.0
4.797	4.5	4.7	4.7	4.7	4.5	4.5
5.085	5.2	5.1	5.2	5.1	5.05	5.0
5.5867	5.35	5.7	5.8	5.9	5.5	5.9
6.101	6.0	6.0	6.1	6.1	6.0	6.0
6.5227	6.5	6.5	6.7	6.5	6.5	6.4
7.254	7.05	7.0	7.1	6.9	7.0	6.9
7.605	7.6	7.5	7.4	7.5	7.2	7.1
8.1607	8.0	7.5	8.0	8.0	7.9	8.0
8.5702	8.0	8.4	8.4	8.3	8.3	8.5
9.0967	9.0	8.5	9.0	8.5	8.5	9.0
9.5355	9.2	9.5	9.5	9.0	9.5	9.5
10.2667	10.1	10.0	10.1	10.1	10.1	10.0
10.764	10.5	10.1	10.5	10.5	10.2	10.2
11.0565	12.0	11.0	11.0	11.0	11.0	11.0
Max. difference from Lüttke-Martius	0.9435	0.664	0.264	0.5967	0.5967	0.564
Min. difference	0.017	0.0	0.028	0.0115	0.0385	0.076
Average difference	0.4802	0.332	0.146	0.304	0.3175	0.320

This instrument and method were tested as follows: (a) Tests of the calibration; (b) estimation of chlorids in aqueous solution; (c) estimation of chlorids in normal urine, undiluted; (d) estimation of chlorids in undiluted urine containing albumin. In all titrations results obtained by the Lüttke-Martius method were taken as the absolute for comparison.

(a) *Tests of the Calibration.*—In comparing by buret readings the calibration of six of these tubes with the table given by Achard and

Thomas, it was found that the difference between the actual and required cubic content of the tube between the marks of the scale was never more than 0.2 c.c. On the whole, the scale marks were found to be accurately placed.

(b) *Estimation of Chlorids in Aqueous Solution.*—The results of determinations of chlorids in aqueous solution by the Achard-Thomas method as compared with the Lüttke-Martius method are shown in Table 6. The Achard-Thomas determinations are seen to vary from the Lüttke-Martius by as much as 0.9435 gm. of chlorids per liter. Readings were obtained between this degree of error and no error. Nearly all readings were too high. The average error of all readings was about 0.3 gm. per liter. As this series of solutions was the same as that used in testing the Strauss tubes, comparison between these results may be made by reference to Table 2. From this it will be seen that the error in the Strauss determinations (0.2 gm. per liter) were considerably less than this, and in no instance were even one-half as great as some of the errors in the Achard and Thomas estimation.

The explanation of these large errors is provided by the character of the chemical reaction and the construction of the tubes. It has long been admitted that the Mohr method for the estimation of chlorids gives too high results, and this may be taken as one factor in the error under consideration. Furthermore, the end-point of the titration reaction is not a sharp one, especially under the conditions of the analysis in the Achard-Thomas tube. The final color of the titration in the tube does not come suddenly, as in the Strauss method, but develops gradually. A good yellow color is obtained in the fluid of the tube long before all of the brownish-red precipitate disappears. Scale readings of varied degrees, therefore, may be made according as the end-point is judged by the permanent yellow color in the fluid (as the directions specify), or by the complete change in the precipitate (as the chemistry of the reaction demands). This introduces another source of error. All my readings were taken when the precipitate changed completely from brown to yellowish white, as I found that this point gave the most accurate results as compared with the Lüttke-Martius figures. In addition, the scale itself is a source of error. It is constructed with relatively few marks, separated by considerable and varying distances on the tube, with no register between them. The readings, few of which corresponded exactly to the marks on the scale, were taken as the nearest approximations that judgment allowed.

(c) *Estimation of Chlorids in Normal Urines.*—Table 7 presents the results obtained by chloridometry performed on the same series of normal urines used for Table 3, for the Strauss tubes. These results are more like the Lüttke-Martius figures than those in the above table when

aqueous solutions of sodium chlorid were used. The errors in some cases are considerable, however, being as high as 1.299 gm. per liter. This is much a larger error than any in the Strauss determinations, and the use of the tubes in these tests exhibit the same difficulties of inconvenient scale, approximate readings, and uncertain end-point as were described above. Their advantage over the Strauss method is that the color of the urine makes no difference at all in the distinctness of the end-color, when finally this is obtained. Estimations by this method can be made somewhat more rapidly than by the Strauss process.

TABLE 7.—ACHARD AND THOMAS TUBES WITH CHLORIDS OF NORMAL URINE

Method	Gm. NaCl Per Liter					
Lüttke-Martius	2.0475	4.9725	6.903	7.722	9.716	11.349
Achard-Thomas —						
Tube 1	2.0	.....	7.0	8.2	8.5	10.05
Tube 2	2.0	4.8	6.8	7.5	9.0	11.0
Tube 3	.....	5.0	6.9	8.0	9.0	10.05
Tube 4	.....	.....	7.0	7.6	8.0	.....
Tube 5	.....	.....	.....	.....	9.0	.....
Tube 6	.....	.....	.....	.....	9.0	.....
Max. difference	0.0475	0.1725	0.103	0.478	1.216	1.299
Min. difference	0.0475	0.0275	0.003	0.122	0.716	0.349
Mean. difference	0.0475	0.10	0.052	0.300	0.866	0.824

(d) *Estimation of Chlorids in Albuminous Urine.*—A series of urines containing the proteins of blood-serum were analyzed for their chlorid content by the Achard-Thomas method. This series was the same as that for the Strauss tubes, shown in Table 4. Chlorid determinations were made on urines to which salt-free protein had been added in quantities up to 27 gm. per liter. The titrations were all within the limits previously discovered to represent the accuracy of these tubes. In no case was the presence of protein found to interfere with the Achard-Thomas process.

Tests of this method show that because of the inconveniences of its irregular scale, and the uncertainty of its end-point, the Achard-Thomas process is capable of giving only widely approximate results. It has the advantage of being rapid and uninfluenced by the color of the urine tested, while it requires only one standardized solution. But its inaccuracies are so great that its value as a clinical method is considerably less than that of the process of Strauss.

CHLORIDOMETRY BY ACHARD-THOMAS METHOD IN GRADUATED CYLINDERS

This method, like that of Strauss, may be simplified further by eliminating the special apparatus, the chloridometer tube, and employing a graduated 50 c.c. cylinder. The titration may be made in one of these

cylinders and the total volume of fluid noted at the end of the analysis. From the amounts of standard silver nitrate solution, the quantity of urine added and their chlorid equivalent, a formula may be derived by which the total volume of fluid at the end of the titration may be converted into grams of chlorid per liter of urine. Into the cylinder 5 c.c. of standardized Achard and Thomas silver nitrate solution are placed. Of this solution, 1 c.c. will precipitate 0.01 gm. NaCl. To this are added 2 to 3 drops of potassium chromate. Urine is then added until the yellow color appears, determining the end-point as in the ordinary manner. From these quantities the following formula is derived:

V = total volume of fluid in cylinder at end of titration ($V - 5$) = amount of urine added.

$5 \times 0.01 = 0.05$ = amount of NaCl precipitable by 5 c.c. of $AgNO_3$ solution.

As the chlorid content of the amount of urine added ($V - 5$) is 0.05 gm. when the end-point is reached, then

$$\text{Grams NaCl per c.c.} = \frac{0.05}{V - 5}$$

$$\text{Grams NaCl per c.c.} \times 1{,}000 = \text{grams NaCl per liter.}$$

$$\therefore \text{Grams NaCl per liter} = \frac{0.05 \times 1{,}000}{V - 5} = \frac{50}{V - 5}$$

Example: Suppose $V = 19$

$$\text{Gm. NaCl per liter} = \frac{50}{19 - 5} = \frac{50}{14} = 3.57.$$

This result corresponds to the figures in the table given by Achard and Thomas.

In applying this method, as described above, or by reversing the procedure, adding the silver nitrate solution to the urine, as in the Mohr technic, unsatisfactory results were obtained. The short scale length and the uncertainty of the end-point make the results of titrations in graduated cylinders quite inaccurate. The readings obtained by the cylinders, however, were as accurate as those by the tubes of Achard and Thomas, and the cylinders have the advantage of providing a scale of more calibrations than that on the tubes. But the errors are so great that they prevent recommendation of the method for clinical use.

CONCLUSIONS

Several simplified methods for estimating chlorids in the urine are described and tested. Of these procedures:

1. The Strauss method is found to be simple and rapid and to give results, which, while not exact enough to serve as a basis for metabolic experiments, are sufficiently accurate for clinical purposes.

2. The Strauss method is directly applicable to both normal and albuminous urine.

3. The Strauss method modified by performing the test in a graduated 50 c.c. cylinder, instead of the Strauss tube, gives results more accurate than the original method, and is a simple R process. A scale is computed for use with such cylinders.

4. The Achard and Thomas method is simple and rapid, but because of uncertain end-point and inconvenient scale, is not sufficiently accurate.

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A PRELIMINARY NOTE ON THE EXCRETION OF INDOL-ACETIC ACID IN THE URINE*

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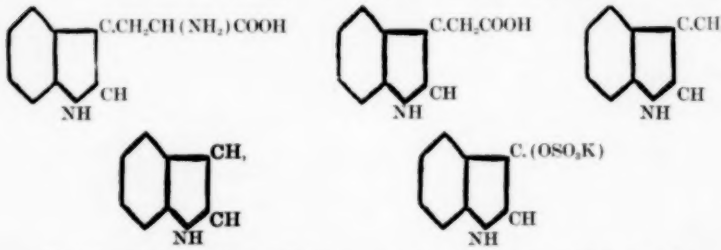
In making metabolism studies in cases of dementia praecox I was struck by the appearance of a red color in the urines which had been treated with strong mineral acids. The red substance was found to be insoluble in ether, chloroform, benzene, toluene and xylene, but soluble in water, ethyl alcohol and amyl alcohol. In amyl alcohol the red substance gave an absorption spectral band between 556 and 570. This compound with these characteristics seems to be identical with the urorosein described by Nencki and Sieber,[1] H. Rosin,[2] C. A. Herter,[3] J. Ph. Staal,[4] L. C. Willard,[5] V. Arnold[6] and A. Ellinger and A. Flammond.[7] According to the work of Herter, the urorosein is formed from indol-acetic acid by the action of a strong mineral acid and an oxidizing agent.

That indol-acetic acid is derived from tryptophan can hardly be doubted, for first the indol group is common to both, and second, tryptophan is abundant in animal tissues and in the daily diet of man. The close chemical relationship between tryptophan, indol-acetic acid, skatol, indol and indican is clearly shown by the following graphic formulas:



Skatol and indol are well known toxic substances, which, in normal metabolism are made non-toxic by their transformation into ethereal sulphates, one of which is indican.

* From the Laboratory of the Illinois State Psychopathic Institute.
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Nencki and Sieber[1] were not able to find indol-acetic acid in the urines of normal persons. Rosin[2] claimed to have found it in every normal urine examined, and in greatest amount when the diet consisted of vegetables. Garrod and Hopkins[8] say that it occurs only occasionally in normal urines. Herter claims that with potassium nitrite and concentrated hydrochloric acid it was impossible to obtain the reaction except in very faint suggestions from any normal urine examined. Nencki and Sieber report the presence of "urorosein" in the urines of patients suffering with osteomalacia, nephritis, typhoid fever, carcinoma of the esophagus, ulcer of the stomach and perityphlitis. They say that 10 per cent. of all diseased persons gave a positive reaction. Herter reported indol-acetic acid in the urine of an under-developed child suffering from a peculiar intestinal putrefaction.

Since the findings have been somewhat contradictory thus far, it was thought best to examine the urines of a number of apparently healthy persons. The urines of ninety-one employees of the Kankakee State Hospital were collected and tested for indol-acetic acid. The individuals were all young, working and apparently in good health. The following tests were made on each urine: Ten c.c. of urine in a test-tube was treated with about 3 c.c. of hydrochloric acid (sp. gr. 1.19) and 2 drops of 0.2 per cent. sodium nitrite. The contents of the tube were mixed and allowed to stand ten minutes. If a pink or red color appeared the test for indol-acetic acid was called positive. The second test was identical with the first, except that no nitrite was used.

TABLE 1.—TESTS ON URINES OF YOUNG, HEALTHY PERSONS

	No. of Cases	Test without O-Agent		Test with O-Agent	
		Number Positive	Per Cent. Positive	Number Positive	Per Cent. Positive
Men	68	5	7.35	13	19.12
Women	23	2	8.70	6	26.09
Total	91	7	7.69	19	21.37

These results indicate that indol-acetic acid may appear in the urine of apparently healthy young people.

As I first noticed indol-acetic acid in the urine of insane patients in quantities relatively very large, an examination was made of a large number of such urines. The results of the tests similar to those described above were as shown in Table 2.

8. Jour. Physiol., xx, 112.

TABLE 2.—TESTS OF URINES OF INSANE PATIENTS

Type of Mental Disorder	No. of Cases	Test without O-Agent		Test with O-Agent	
		No. Positive	Per Cent. Positive	No. Positive	Per Cent. Positive
Organic brain disease (not differentiated)	7	1	14.29	2	28.58
General paralysis of the Insane	21	2	9.52	4	19.04
Senile dementias	16	2	12.50	10	62.50
Infective exhaustive psychoses	3	1	33.30	3	100.00
Intoxication psychoses (alcohol and morphin)					
Chronic	14	1	7.14	4	28.57
Acute	6	1	16.67	2	33.33
Total	20	2	10.00	6	30.00
Dementia praecox group					
Hebephrenic	42	4	9.52	15	35.71
Katatonic	12	5	41.66	10	83.33
Paranoid	18	2	11.11	10	55.55
Not differentiated	102	8	7.84	48	47.06
Total	174	19	10.92	83	47.70
Manic depressive group					
Depressed	16	1	6.25	6	37.50
Excited	17	2	11.76	4	23.52
Remission	2	0	....	0	....
Total	35	3	8.57	10	28.57
Involutional melancholias	5	0	....	2	40.00
Psychoneuroses	2	0	....	2	100.00
Paranoic states	4	1	25.00	3	75.00
Psychopathic personalities	3	0	....	1	33.33
Epileptic psychoses	42	3	7.14	15	35.71
Defective mental development	26	3	11.54	15	57.70
Unclassified cases of insanity*	132	18	13.64	65	49.24
Tuberculosis in insane individuals†	26	3	11.54	10	38.46
Very inactive insane individuals	25	4	16.00	13	52.00
Total insane individuals tested	490	55	11.22	211	43.06

* Old cases which are largely cases of dementia praecox undoubtedly.
† These individuals were also included in the different psychosis groups.

Of the 490 urines from insane patients, 43.06 per cent. contained indol-acetic acid as compared with 21.37 per cent. in the case of normal urines. Such a large difference indicates a relation between indol-acetic acid excretion and some condition frequent among the insane. The data in Table 3 exclude the validity of the claim that the age factor is an influential one.

In many cases the number of patients of a certain type of psychosis is too small for the percentages of positives to be of any considerable value alone. It will, however, be noted that where the psychoses seem to arise on a basis of constitutional inferiority, the percentage of positive reactions is generally relatively high. The best example of this is found in the case of the dementia praecox group with 174 cases and 47.7 per cent. positive, and in the case of the mental defectives with twenty-six cases and 57.7 per cent. On the other hand, the best examples of low percentages are found in the group of general paralysis with twenty-one cases and 19.04 per cent. positive, and the intoxication group with twenty cases and 30.00 per cent. positive. The intoxication psychoses and general paralysis of the insane may both be considered as more definitely acquired conditions, since they require the presence of some exogenous

TABLE 3.—AGE FACTOR IN INDOL-ACETIC ACID EXCRETION

	No. of Cases	Test without O-Agent		Test with O-Agent	
		No. Positive	Per Cent. Positive	No. Positive	Per Cent. Positive
Insane individuals of ages between—					
15-25	32	.....	.....	14	43.75
25-40	165	.....	.....	76	46.06
40-60	203	.....	.....	93	45.85
60—above	57	.....	.....	23	40.35

factor. Dementia praecox and defective mental development, on the other hand, are generally believed to be more definitely constitutional in nature. This apparent relation between constitutional inferiority and indol-acetic acid excretion is strengthend when we compare the percentage of positive among the cases belonging to the dementia praecox group with the percentage of positives among the insane other than dementia praecox. This point is clearly shown in Table 4.

There are at least two possibilities to be considered in connection with the occurrence of indol-acetic acid in the urine. First, like homogentisic acid and cystin, it may indicate a constitutional disorder of metabolism. The figures given above tend to suggest that this is true, although it cannot yet be considered as proven. Secondly, it may result from a particular kind of intestinal putrefaction, for Hopkins and Cole[9] have

9. Jour. of Physiol., 1903, xxix, 29-451.

shown that indol-acetic acid can be produced *in vitro* from tryptophan by bacterial growth. Work along these lines is being actively carried on at the present time in this laboratory.

TABLE 4.—Comparison of Positive Cases Among Cases of Dementia Praecox and Other Forms of Insanity

	No. of Cases	No. Positive	Per Cent. Positive	No. Positive	Per Cent.
Sane persons apparently healthy	91	7	7.7	19	21.4
Insane persons, exclusive of dementia praecox and unclassified groups	184	18	9.8	63	34.2
Dementia praecox group	174	19	10.9	83	47.7

Another point also needs further study. It will be noted that to some urines an oxidizing agent had to be added in order to convert the indol-acetic acid to urorosein, whereas in others no such addition was necessary. Herter commented on this fact and showed that a positive urorosein reaction might appear without adding an oxidizing agent if the urine containing indol-acetic acid were allowed to stand twelve to twenty-four hours, and claims to have proven that this was due to the growth of nitrifying organisms whereby the necessary nitrite was provided. This certainly is not necessary in all cases, for a positive reaction without the addition of any oxidizing agent has been obtained in specimens immediately after they are voided. It therefore remains to explain the presence of an oxidizing agent in some urines. This point is also being investigated in this laboratory.

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